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TEMPERATURE REQUIREMENTS FOR GERMINATION OF SPORES OF CRONARTIUM FUSIFORME

PAUL V. SIGGERS[1]

(Accepted for publication July 22, 1947)

In the southern half of the Gulf States the pycnial and aecial stages of *Cronartium fusiforme* (Arth. & Kern) Hedgc. & Hunt, the cause of fusiform rust, most commonly occur on elongated cankers on woody parts of loblolly pine (*Pinus taeda* L.) and slash pine (*P. caribaea* Morel.), and less frequently on longleaf pine (*P. palustris* Mill.). The uredial and telial stages occur on the leaves of oaks, chiefly species of the pointed-leaf or black oak group.

This disease has become increasingly prevalent in the lower Gulf region during the last 35 years. Since loblolly and slash pines are less resistant than longleaf pine to infection, wider distribution and intensification of the disease may be ascribed in part to the natural encroachment of the more rust-susceptible pines and oaks on lands originally occupied almost exclusively by longleaf pine.

In recent years the reforestation program for the lower South created a strong demand for slash pine planting stock (8). By 1940, the estimated annual production of slash pine at seven nurseries, exclusive of planting stock produced at two U. S. Forest Service nurseries, was 37 million seedlings, compared with 11,000,000 loblolly pine and 2,600,000 longleaf pine seedlings. Since slash pine is highly rust-susceptible, a problem of controlling the fusiform rust soon developed in southern pine tree nurseries. Sleeth (9) reported that losses of slash and loblolly pine seedlings, caused by the disease, exceeded 4,000,000 in 1938 and amounted to 3,000,000 in 1939.

Bordeaux spray treatments to control the disease have not given consistently favorable results. Control measures at one nursery showed little worthwhile benefit in 1939 and 1941. Results of the treatments were favorable in 1940 (9) but less favorable in 1942.

Knowledge of environmental factors affecting rust infection is of fundamental importance in control of the disease in forest tree nurseries. For example, spore germination is necessary for infection, therefore, a better understanding of the effect of temperature on germination may aid in the development of more effective spray schedules for control of the disease in nurseries. Knowledge of the temperatures that inhibit spore germination would be of great aid in studying and interpreting the results of inoculations. The effect of temperature on spore germination was studied during the spring and fall of 1945 and during the spring of 1946.

METHODS AND MATERIALS

In all tests spores were germinated in direct contact with distilled water or in a moisture saturated atmosphere. Van Tieghem cells were used in

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germination tests of aeciospores, urediospores, and teliospores. Two cells were prepared for each glass slide, by fastening glass rings (3 mm. high by 15 mm. diameter) to the slide with high-melting-point paraffin. As an added precaution against loss of water, the Van Tieghem cells were sometimes placed in Petri dishes lined with moist filter paper. In some tests the cells were placed inside large iceless refrigerators (5), instead of Petri dishes. Sporidia were germinated in water under cover slips on glass slides.

Spore Germination Technique

Different methods were devised for germinating different types of spores. With aeciospores and urediospores, a drop of water was centered on a cover slip and a small fragment from a young oak leaf was placed on top of the water. A small quantity of spores was transferred to the drop by the point of a sterile inoculating needle. The cover slip bearing the droplet was then quickly inverted over the ring. The spores floated more or less freely on the surface of the water.

The telium was the unit of observation in germination tests involving teliospores, because teliospores cannot be separated from the telium-matrix and still remain viable. A drop of water was placed in the bottom of the Van Tieghem cell. Oblong sections of oak leaves, with telia, were cut slightly longer than the diameter of the ring so that the leaf segment, when mounted in the cell, would arch above the water with the telia pointing downward over the drop. A glass cover slip closing the cell prevented excessive loss of water.

Sporidia were obtained by placing telia in Van Tieghem cells overnight at temperatures near the optimum for teliospore germination. In the morning, after the sporidia had been formed, telia were cut off and mounted on a glass slide in a drop of water under a cover slip. The slides were then tapped lightly to detach mature sporidia from the promycelia.

Duration of Germination Tests

Germination tests with aeciospores, urediospores, and teliospores started in late afternoon, usually about 5 p.m. and continued for 15 hours. Germination tally of aeciospores and urediospores, starting the following morning, continued sometimes until the 16th hour. One hundred to 150 spores were counted in each test. Germination of sporidia lasted for 5 or 6 hours; however, 3 tests ran 9 hours. Sixty to 100 spores were counted in tests with sporidia.

Temperature Equipment

For low-temperature tests an electric refrigerator was available and for high-temperature germination an electric incubator was used. For intermediate temperatures, home-made iceless refrigerators were constructed and mounted in shallow vats filled with water and kept moist by water dripping from above. Maximum and minimum thermometers recorded the range of temperatures in tests within the electrical refrigerator and in-

cubator. A Friez hygrothermograph recorded humidity and temperatures within the iceless refrigerator. The accuracy of this instrument was checked about once a week with a Friez psychrometer.

The refrigerator and the incubator could not be classed as biological constant-temperature equipment and there was no temperature control inside the iceless refrigerators. Temperatures within the humidity chambers varied with that on the outside, except that during the warmer periods of the day it was usually 3° to 7° C. cooler inside the chambers than outside.

Source of Spores Used and Locality of Collection

Aeciospores came from 6 fusiform cankers on *Pinus caribaea* and *P. taeda* from Harrison, Jones, and Stone Counties in southeastern Mississippi and from *P. rigida*[2] Mill., Bent Creek, N. C.

Urediospores were obtained by inoculating leaves of seedlings of *Quercus nigra* L. and from 2 field collections on *Q. phellos* L. near Pearlington, Mississippi, dated March 24, 1945.[3]

Teliospores originated from leaves of seedlings of *Quercus nigra* that had been inoculated with aeciospores of *Cronartium fusiforme*.

Sporidia came from teliospores that, in turn, had resulted from inoculation of leaves of *Quercus nigra*. All inoculations were made in a greenhouse near Saucier, Miss.

PRELIMINARY EXPERIMENTS

Preparatory to study of the effect of temperature on germination of rust spores, the minimum period required to start germination and the germinative capacity with reference to age of spore collection and storage conditions were investigated. There was no information available on the time required to initiate spore germination, and observations in the field indicated that the yellow-ochre color associated with fresh aeciospores disappeared in about 5 weeks. When this color change occurred it was thought that the spores were no longer viable.

Minimum Period Required to Start Germination

Aeciospores. In five tests with as many different collections of aeciospores, the minimum period required for the germination of aeciospores varied from 2¼ hours to about 4 hours (Table 1).

Urediospores. The minimum period for the initiation of germination was determined for two lots of urediospores. A day-old collection of spores from greenhouse inoculation of a water oak (*Quercus nigra*) seedling was

[2] Received from Dr. George H. Hepting, U. S. Forest Pathology Field Laboratory, 223 Federal Bldg., Asheville, N. C.

[3] Urediospores from natural infection were resorted to because uredial sori were seldom produced abundantly by inoculation. The writer believes that the uredial sori collected in March, 1945 were those of *Cronartium fusiforme* because the ranges of *Pinus echinata* Mill. and *P. virginiana* Mill., the more common hosts for *C. cerebrum* (Peck) Hedge. and Long (3) do not extend as far south as Pearlington, Miss.; more than 99 per cent of the cankers in the locality of collection were fusiform, in contrast to the globose canker formed by *C. cerebrum*, and also *C. fusiforme* fruits earlier in the growing season than *C. cerebrum*. Peak development of the uredial stage took place earlier in 1945 than in any other year of record.

incubated at 25°–26° C. Between 6 and 7 per cent of the spores germinated in 1 hour. Spores from a 7-day collection on willow oak (*Q. phellos*) started germination after incubation at 22°–23° C. for 1 1/3 hours.

Teliospores. Nongerminated teliospores of 29-day-old telia[4] from greenhouse inoculation were held at temperatures fluctuating from 17°–23° C. and examined at 3-hour intervals starting 9 a.m. April 19, 1945. Microscopic examination at 6 p.m. showed teliospores were germinating but not abundantly. Under these conditions, nine hours was the minimum period for germination of teliospores. By 9:15 p.m. of the same day teliospores were germinating from the tip to the base of the telium and sporidial "spore prints" (Fig. 1, A) on the surface of the drop of water contained clusters of 50 to 150 sporidia. Obviously, abjection of sporidia had set in just a short time before.

The effect of shorter exposures to temperature and moisture conditions favorable for teliospore germination was studied. Teliospores of 17-day-

TABLE 1.—*Minimum period required for germination of aeciospores of Cronartium fusiforme*

Source of spores	Age of collection (Days)	Temperature (°C.)	Duration (Hours)	Results
Pinus taeda	37	23	1¾	Negative
P. caribaea	36	21–23	2½	Negative
P. rigida	23	23	2¼	Positive—1 pct.
P. caribaea	51	23	3¾	Positive—trace
P. taeda	44	23	5¾	Positive—5 pct. germinating between 3¾ and 5¾ hrs.

old telia were exposed to favorable temperature and moisture conditions for periods of less than 9 hours on 3 successive days and stored at night in a refrigerator, at 11°–15° C. Under these conditions, a small number of sporidia were found at the end of the third day after a cumulative exposure of 23 hours to favorable environmental conditions.

Sporidia. Sporidia obtained in May from 14-day-old telia and incubated for 3 hours at 17°–18° C. started germination. Sporidia from the same source, incubated at 21°–22° C., started germinating in 2½ hours. Twenty-eight per cent of the spores had germinated by the fourth hour. In a third test, with telia about 38 days old, sporidia incubated at 28°–29° C. started germination in 2¼ hours. Seven per cent of these spores had germinated by the fifth hour.

Germinative Capacity with Reference to Age of Collection and Storage Conditions

Aeciospores. Aeciospores from a canker on slash pine, collected March

[4] The age of telia in days was counted starting from the first day that primary sori from greenhouse inoculations became visible to the unaided eye. After inoculation with aeciospores in mass, the shortest incubation period was found to be 6 days, yet for about 10 days thereafter additional telial sori—also primary sori as they resulted from a single application of aeciospores—showed up on the same leaf.

14, 1945 and stored below 10° C., maintained germinative capacity at high level for 47 days. Aeciospores from loblolly pine, collected the same day and stored under similar conditions, maintained good viability for 76 days. Spores from this lot germinated (0.5 per cent) on October 8 and were used successfully for inoculation on October 12, 211 days after collection. Some lots of aeciospores remain viable for months, at low temperatures, but

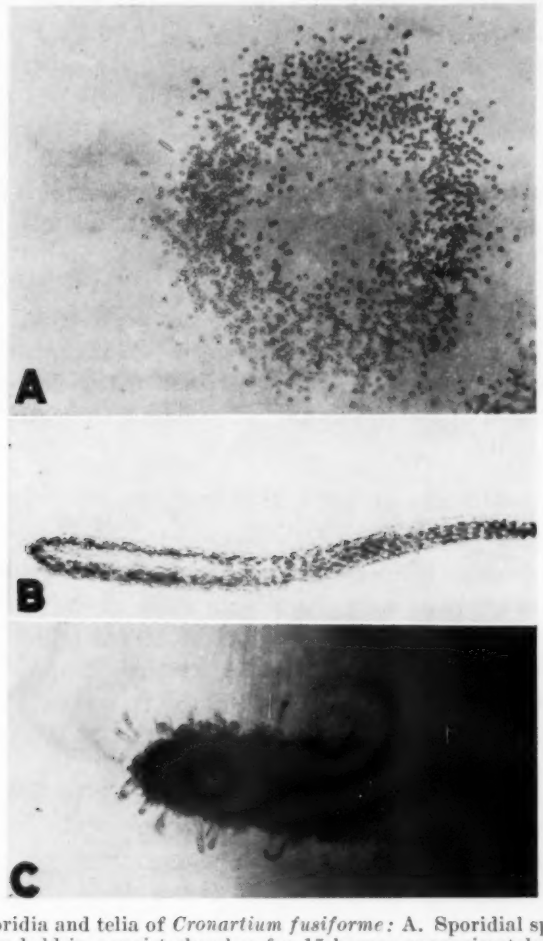

FIG. 1. Sporidia and telia of *Cronartium fusiforme:* A. Sporidial spore print formed from telial column held in a moist chamber for 15 hours; approximately 1600 sporidia in the cluster that was formed on a droplet of water. ×65. B. Telium with nongerminated teliospores, obtained by inoculation of oak leaves with aeciospores on April 30, 1945. Photographed June 14, 1945. ×45. C. Apical end of a telium with germinating teliospores. ×85.

germinative capacity is reduced as the storage period lengthens. The first change in color of aeciospores, from yellow-ochre to gray, was found to be associated with a loss in germinative capacity.

Urediospores. Five per cent germination was obtained with urediospores collected March 24, 1945 and stored at 4° to 10° C., when tested on October 5, 1945. These were germinated after 15 hours exposure to 17°–22° C. The

same spore collection was used successfully for inoculation on Nov. 2, 1945, 223 days after collection.

Teliospores. Although dormant teliospores (Fig. 1, B) withstand unfavorable environmental conditions, germinative capacity is reduced if the telia are subjected to high temperatures that approach lethal temperatures. Teliospores of 29-day-old telia formed a few sporidia in one of two Van Tieghem cells on June 7, 1945. In the next test, on June 9, teliospores failed to germinate after 23 hours at 23°–24° C. In a final test, started June 12 at 6 p.m. and lasting 70 hours, with the temperature at 23°–24° C., sporidia were deposited in one of four cells. This indicates that the fungus was still alive in one of the leaf segments and that young teliospores, developing at the base of the telial column, had germinated during the relatively long period of exposure at favorable environmental conditions. Probably most of the fungus in the leaf tissue had been killed by high temperatures and low humidities that prevailed prior to the test. Daily maximum greenhouse temperatures exceeded 32° C. during the week starting June 3 and exceeded 35° C. during the following week.

Sporidia. Attempts to store sporidia were not successful. All observations indicate that sporidia are short-lived, and that high temperatures or moisture conditions unfavorable for germination quickly cause loss of germinative capacity.

THERMAL LIMITS AND OPTIMUM TEMPERATURES FOR GERMINATION

Since the spore germination data presented were based on fluctuating temperatures, the mean temperature was determined for each test from the range. Most observations were made with Fahrenheit thermometers but, for convenience, the data are presented in the Centigrade scale. The thermal limits and optimum temperatures recorded for spore germination, although not shown in table 2, were obtained from graphs based on the Fahrenheit scale. In converting to degrees Centigrade, fractions in °C. were rounded off to the nearest whole number.

Aeciospores. Eighty-two tests were run with aeciospores from several sources. The lower and upper thermal limits for germination are slightly below 11° C. and 29° C. The optimum temperature for germination is about 21° C. Good germination occurred over a range of average temperatures from 17° C. to 22° C. (Table 2).

Urediospores. The results from 54 germination tests with urediospores are given in table 2. The lower and upper thermal limits for germination were found to be slightly above 8° C. and at 29° C., respectively. The optimum temperature for germination is about 18° C. The data (Table 2) indicate that urediospores germinated well over a range of average temperatures from 15° to 20° C. Urediospores germinate at lower temperatures than aeciospores and have a lower optimum temperature.

Teliospores. It was not possible to express the germination of teliospores on a percentage basis. Germination failed in 8 tests below 15° C. and in 7 tests above 26° C. The optimum temperature for germination, though

not determined, is thought to be about 21° C. The optimum temperature for the germination of teliospores would be that temperature which induces germination (Fig. 1, C) and formation of sporidia in the shortest time. Teliospores started germination in a minimum of nine hours in weak diffuse light with temperature fluctuating between 17° and 23° C.; however, observations on this point were too few to permit drawing any conclusions.

Sporidia. The lower limit for germination of sporidia was defined by the failure of spores to germinate at an average temperature of 13° C. and 4 per cent germination at an average temperature slightly below 14° C. The

TABLE 2.—*Germination of aeciospores, urediospores, and sporidia of Cronartium fusiforme at different average temperatures*

Temperature class centers	Aeciospores			Urediospores			Sporidia		
	No. of tests	Mean germination	Range in germination between tests	No. of tests	Mean germination	Range in germination between tests	No. of tests	Mean germination	Range in germination between tests
°C.		Per cent	Per cent		Per cent	Per cent		Per cent	Per cent
7	1	0.0	.....	2	0.0	.....	.....	.....	.....
9	6	0.0	.....	7	6.7	3–11	1	0.0	.....
11	5	2.7	0–10	1	43.0	.....	1	0.0	.....
13	8	18.6	3–65	1	12.0	.....	3	2.6	0–4
15	5	65.8	31–86	5	75.0	32–90	.....	.....	.....
17	10	67.7	40–91	5	85.4	73–92	1	61.0	.....
19	9	76.8	61–98	4	76.7	73–82	9	60.1	28–80
21	9	83.1	53–97	7	75.0	60–98	4	69.2	48–92
23	6	73.3	49–95	10	56.7	25–84	6	78.1	46–93
25	6	43.5	15–69	6	34.0	19–54	4	44.0	13–82
27	10	9.2	1–17	3	2.3	0–4	7	17.7	4–50
29	7	2.9	0–9	2	3.0	0–6	6	5.0	3–9
31	.....	.....	.....	1	0.0	.....	.....	.....	.....
Total	82	.....	.....	54	.....	.....	42	.....	.....

upper limit for germination appears to be a little above 29° C. average temperature. The optimum temperature for germination is 22° C.

DISCUSSION

The minimum average temperature for germination of aeciospores was slightly below 11° C. and the maximum average temperature was 29° C. Doran (2) and Hirt (4) working with aeciospores of *Cronartium ribicola* Fisch. v. Waldh. found the lower and upper thermal limits for germination to be 5° C. and 28° C., respectively. Germination of fusiform rust spores above 27° C. is chiefly of academic interest because in the Gulf region outdoor temperatures above 27° C. are always associated with periods of relatively low humidity—too low to permit germination of rust spores. Furthermore, reduced hyphal growth and apical curling of the germ tubes were commonly observed in tests in which the average temperatures ranged from 26° to 29° C. Inoculation tests confirmed the results from germination

studies. In 14 successful inoculations, temperatures never exceeded 26° C. during the first 48 hours, except in one test 27° C. was recorded between the 17th and 18th hours. This was followed by a steady drop to 18° C. during the next 17 hours. Although aeciospores and urediospores of *C. fusiforme* germinated at 27° C. it seems probable that infection would not occur at temperatures maintained at 27° C.

The study indicated that teliospores withstand temperatures that would be lethal to other types of spores. For example, exposure of aeciospores to 27° C. for 15 hours reduced germination to 1 per cent; thereafter, only 1 per cent germinated when exposed to lower laboratory temperatures for 24 hours. In a parallel test at 19° C., 98 per cent germinated in 15 hours. On the other hand, teliospores that were formed March 6, 1945, and were exposed to 29°–34° C. for 5½ hours on May 1, 1945 and to 27°–32° C. on May 7, germinated and produced sporidia on May 8.

Both aeciospores and urediospores germinated in water and it is conceived that an adequate amount of water vapor in the air would permit germination of urediospores. The locally restricted pattern of secondary uredial sori sometimes noted on leaves, extending downward from the vicinity of a primary sorus to lower parts of the same leaf surface, strongly implies that water served to distribute the spores from the primary sorus to lower areas of the leaf and that infection occurred only where the droplet or film of water had been. It is generally known that a practically saturated atmosphere is necessary for germination of teliospores of species of *Puccinia*, as indicated by Clayton (1) and Maneval (7) and the moisture requirements for germination of teliospores of *Cronartium fusiforme* appear to be equally high. Both teliospores and sporidia germinated in moisture-saturated atmosphere, and it is thought that teliospores germinate only in this way. Teliospores of telia immersed in water overnight for 15 hours did not germinate. Sporidia that had been deposited on a glass slide in an iceless refrigerator became plasmolized a few minutes after they had been removed to relatively dry atmosphere in an open greenhouse.

Spring spores produced by *Cronartium fusiforme* develop relatively early in the season and are ready to germinate very soon after they are formed. Aeciospores, for example, may infect as early as February. Near Pearlington, Hancock Co., Miss., telial columns about 1 mm. long were found March 15, 1944 on leaves of an oak growing beside a loblolly pine with numerous fruiting fusiform cankers. Inasmuch as the minimum period from inoculation to the appearance of primary telial sori is 6 days, at best, and the average daily growth of a telium is about 100 μ, these telia originated from infection that occurred late in February.[5] Uredial sori appear most abundantly in early April. Primary telial sori may appear two or three days after the first uredial sori. Field observations by Sleeth (9) and by

[5] Teliospores developed and germinated in a minimum of 8 days from inoculation of oak leaves with aeciospores. Most of the inoculations resulted directly in telial sori. Retention of oak seedlings in weak diffuse light in iceless refrigerators probably affected photosynthetic activity adversely, resulting in near suppression of the uredial generation (10).

the writer, in the region where *C. fusiforme* is most abundant, indicate that maximum production of sporidia by this species of rust takes place in April or in May, and that infection, as a seasonal process, is essentially compieted by the latter part of June.

In the infection of pines, the moisture-temperature-time relationship involved in the germination of teliospores, the discharge of sporidia, and their germination are significant. With favorable temperature and moisture conditions, 9 hours are required to start germination of teliospores, 3 hours are needed for development of sporidia, and 6 hours elapse before the abjected sporidia germinate in quantity. In other words, starting with nongerminated teliospores, a minimum of 18 hours, with temperatures fluctuating between 16° and 26° C. and humidity maintained close to the moisture-saturation point are needed for abundant infection on pines.

In nature, such conditions are rather infrequently encountered. The spring of 1938, however, was an unusually favorable season for rust infection. The average percentage of cankered slash pines in 6 of 7 nurseries, from surveys made by Lamb and Sleeth (6), was greater in 1938 than in 1937 or in 1939—usually several times greater. Meteorological records from a weather station in Harrison County, Mississippi, indicate that eight times, between March 28 and May 8, 1938, temperature and moisture conditions favorable to sporidial production and germination prevailed for 18 hours or longer. At no time in 1937, and only once in 1939 did similar conditions prevail during the same period. This explains why the bulk of natural rust infections appear to be confined to certain years, during which the conditions favoring production, dissemination, and germination of inoculum briefly but frequently prevailed, resulting in relatively heavy infection in those years.

Control measures in forest tree nurseries where the fusiform rust has consistently caused loss of planting stock should be timed with reference to periods when the infection hazard is high. In nature, sporidia are produced in large numbers, if prolonged dry weather in April and May is followed by a period of rainy weather for a day or more. Spraying a day before a rainy period would prove more effective than application of a fungicide 3 or 4 days before the humid period. Timing the treatments in this manner should be feasible, with long-range weather forecasts available. Since germination of sporidia, and, therefore, infection of pine seedlings does not occur at 13° C., spraying could be deferred as long as the daily average temperature is not in excess of 13° C.

SUMMARY

The effect of temperature on germination of four types of spores produced in the spring by *Cronartium fusiforme* (Arth. & Kern) Hedgc. & Hunt, the cause of fusiform rust, has been studied. In all tests spores were germinated in distilled water mounts or in an atmosphere maintained at the moisture saturation point.

The minimum period required to start germination was investigated preparatory to a more detailed study of the effect of temperature on the process. Fresh aeciospores started germination in 2¼ hours at 23° C. A day-old collection of urediospores started germination in less than an hour at 25° C. Teliospores started to germinate in a minimum of 9 hours at 17° to 23° C., and sporidia, in 2¼ hours at 28° to 29° C.

Germinative capacity of a spore lot depends chiefly on age of the collection and storage conditions. Storage at low temperature, below 10° C., kept germinative capacity of aeciospores at high level for a maximum of 76 days. Urediospores stored under similar conditions were successfully used for inoculation 223 days after collection. Teliospores germinated after exposure to fluctuating temperatures in a greenhouse from March 6 to May 8, 1945. Attempts to store sporidia were not successful.

The lower and upper thermal limits for germination of aeciospores are slightly below 11° C. and 29° C. The optimum for germination is about 21° C. For urediospores, the lower and upper thermal limits for germination are slightly above 8° C. and at 29° C. Urediospores germinate at lower temperatures than aeciospores and have a lower optimum temperature for germination. The lower and upper limits for germination of teliospores are approximately 15° C. and 26° C. The optimum temperature for germination of teliospores was not determined. Sporidia germinate between 13° and 14° C., with the upper limit a little above 29° C. and the optimum temperature at 22° C.

Timing the spray treatments with reference to periods of high infection hazard and deferring control measures as long as daily average temperatures lie below the minimum thermal requirements for germination of sporidia should aid in the preparation of more effective spray schedules for control of the disease in forest tree nurseries.

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CORRELATED RESISTANCE OF LEAVES, COTYLEDONS, AND STEMS OF CUCUMIS MELO L. TO CANTALOUPE POWDERY MILDEW (ERYSIPHE CICHORACEARUM DC.)

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(Accepted for publication August 20, 1947)

In a study designed to determine the number and quantitative effects of the genes for resistance to powdery mildew (*Erysiphe cichoracearum* DC.) in *Cucumis melo* L., it has become important to know whether the various aerial organs of the plant (leaves, cotyledons, and stems) are equally susceptible to attacks by the fungus. It is obvious to the experienced observer that in susceptible varieties leaves, cotyledons, and stems are attacked with equal severity. It is also obvious that in strains with a very high level of resistance leaves, cotyledons, and stems are equally resistant to attacks by the fungus. When a cross is made between a very susceptible line and one that is highly resistant, our problem is to determine whether or not the aerial portions of the plants in the F_2, F_3, and subsequent progenies will be attacked uniformly. If the severity of mildew infection is found to be closely correlated on leaves, cotyledons, and stems of an individual, it would seem safe to assume that the same set of genes for susceptibility is effective throughout the entire aerial portion of the plant. However, should the results indicate that the degree of infection on the leaf was not necessarily an indication of infection to be expected on the stems and cotyledons, or *vice versa*, some other explanation would have to be sought. There is the possibility that such behavior might indicate that different organs of the same plant are differentially susceptible to the fungus, or that different biotypes of the fungus exist which attack the several aerial portions of the plant with an unequal measure of success.

EXPERIMENTAL OBSERVATIONS

In order to examine the question of differential susceptibility of the various organs of the same plant, a considerable number of individuals in several F_3 families were scored for resistance to powdery mildew. The reaction of leaves, cotyledons, and stems of each plant was recorded. The technique of inoculation was the same as that used in previous work.[3,4] As soon as the first leaf unfolded, the plants were placed in a glass-sash chamber and inoculated by blowing conidia from heavily infected leaves into the chamber. This method has proved effective in obtaining a uniform, severe infection on all aerial portions of susceptible plants. Disease symptoms were recorded 16 days after inoculation.

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[3] Pryor, D. E., and T. W. Whitaker. The reaction of cantaloupe strains to powdery mildew. Phytopath. **32**: 995–1004. 1942.

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A total of eight different F_3 families were examined. The number of individuals scored in each family ranged from a low of 19 up to 175 plants in the largest family. Of the eight F_3 families sampled, two were highly resistant, two were very susceptible, and the remainder were approximately intermediate in their reaction toward the parasite. It is thus evident that a fair sample of the variability to be expected of the segregation from the original cross was tested.

Since the problem to be solved is one of determining to what extent low infectibility (or resistance) of one plant part indicates probable infectibility of another, it seems proper to combine the data from the eight families, in order to obtain r values for the comparisons as a whole.

The scores were converted to an arbitrary numerical scale[5] and the correlation coefficients calculated for leaves and stems, leaves and cotyledons, and cotyledons and stems (Table 1). The significance of the correlation coefficients for the several comparisons was evaluated.

TABLE 1.—*Correlation coefficients for mildew infection on the aerial parts of cantaloupe plants*

Number of plants scored	Correlation Coefficients between:		
	Leaves and stems	Leaves and cotyledons	Cotyledons and stems
859	0.818**	0.838**	0.866**

** Significant at the 1-per cent level.

It is evident from the results that there is a high degree of correlation between the severity of mildew infection on the several aerial portions of the plant.

Since the correlations derived in this study are high, it might be helpful to know whether the examination of the leaves would provide a fairly reliable index of the severity of infection on cotyledons and stems. A value of r for this correlation can be calculated by treating the data in a slightly different manner. Assuming that the best estimate of the severity of infection is obtained from the average of the sum of the effects on leaves, stems, and cotyledons, then the estimate of the severity of infection on the leaves, compared with that from the composite picture, yields a value of $r = 0.940$. Thus about 88 per cent of the possible information can be obtained by examination of the leaves alone. This means that in most cases severity of infection on the leaves is a good indicator of the mildew symptoms to be found on cotyledons and stems.

[5] In rating the amount of powdery mildew, the following scale has been used. Type 0—No mycelium evident to the naked eye. Type 1—Only 1 to 3 small colonies developed. Type 2—Little mycelium developed and few conidia formed. Type 3—Mycelium sparsely covers part of all of the leaf; sporulation somewhat suppressed (medium infection). Type 4—The mycelium entirely covers the leaf. Sporulation abundant (severe infection). Type A—Used in connection with the several reaction types to indicate stem cracking and necrotic or chlorotic spotting on leaves and cotyledons. In converting to a numerical scale, Type 0 = 1, Type 0A = 2, Type 1 = 3, Type 1A = 4, etc.

If these eight families are analyzed independently, it is found that those with a very high level of resistance are apt to show less correlation of mildew severity symptoms on the several organs than those that are intermediate or susceptible in their reaction.

DISCUSSION AND CONCLUSIONS

The experimental observations lead to the conclusion that there is apt to be a high degree of correlation between the severity of powdery mildew symptoms on the leaves, cotyledons, and stems of the same plant; *i.e.*, moderate infection on the leaves indicates a corresponding degree of severity on cotyledons and stems. The same situation holds with respect to very susceptible leaves and highly resistant ones.

In general, observations of natural infection of cantaloupes with powdery mildew in the field tend to confirm conclusions arrived at on the basis of our greenhouse study. Very susceptible varieties seem to be attacked with equal severity on leaves and stems. However, progenies with a fairly high level of resistance very often produce plants in which the mildew colonies are present only on the leaves. In such cases it is possible that the stems escaped infection, or were infected only lightly, or perhaps became infected at a later date than the leaves.

There is no evidence from these experiments for the presence of more than one biotype of the powdery mildew fungus. Likewise, there is no evidence to indicate that different organs of the same plant are differentially susceptible to attacks by the parasite. The genes for susceptibility seem to be equally effective throughout all the aerial portions of the plant.

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STEM ROT OF DIEFFENBACHIA PICTA CAUSED BY PHYTOPHTHORA PALMIVORA AND ITS CONTROL

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INTRODUCTION

Potted plants of two varieties of *Dieffenbachia picta* Schott (the regular or dark green type and one with light green foliage known as Rudolph Roehrs), prized by the florists' trade for their attractive, variegated leaves, have been seriously affected in recent years by a stem rot in commercial greenhouses in San Francisco.

This paper discusses the symptoms of the disease, the causal organism, and recommendations for control.

SYMPTOMS OF THE DISEASE

Under conditions favoring natural infection in local commercial greenhouses, the first visible symptom on young or small, as well as on older or

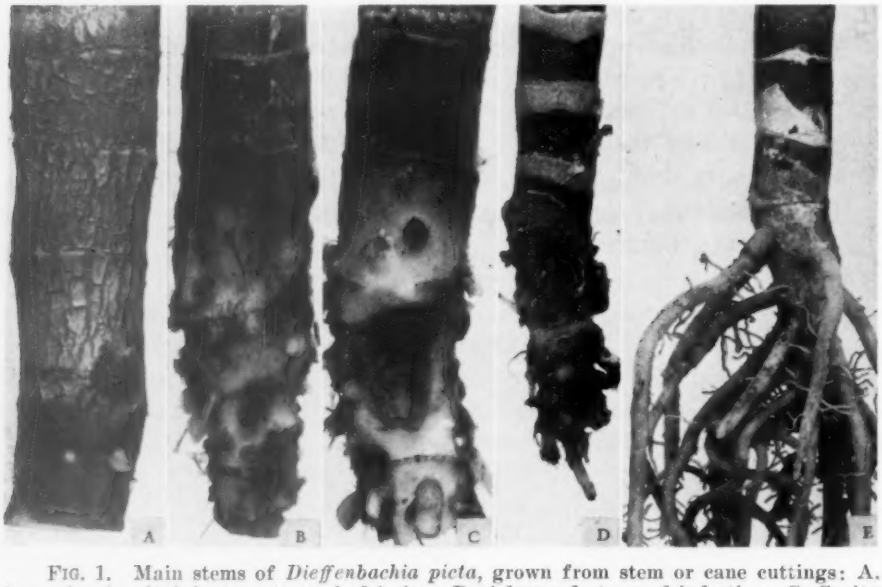

FIG. 1. Main stems of *Dieffenbachia picta*, grown from stem or cane cuttings: A. Stem showing incipient, water-soaked lesion; B. Advanced stage of infection; C. Cavity formed by collapse of internal tissues; D. Infected stem immediately preceding lodging; E. Healthy stem.

larger plants, consists of a small, irregular-shaped, water-soaked lesion on the main stem at the soil level (Fig. 1, A). Usually the lesion increases in size very rapidly and utimately may extend ¼ to 1 inch above and below the

[1] Joint contribution from the Division of Plant Pathology, California Agricultural Experiment Station and the Department of Botany, Missouri Agricultural Experiment Station.

soil level. As infection spreads internally, the invaded stem tissues become soft and watery. Coinciding with infection of the basal part of the main stem of young plants (arbitrarily, less than ½ inch in diameter), the leaves and petioles usually turn yellow and wilt very suddenly. Within a day or two, the main stem breaks at the point of infection, falls, and the plant dies (Fig. 4, A, B). In older or larger plants (whose main stem exceeds ½ inch in diameter), invaded tissues collapse, forming a brown cavity (Fig. 1, B, C, D) which, as it continues to enlarge, weakens the structural stability of the main stem and soon causes breakage and lodging (Fig. 2). In contrast

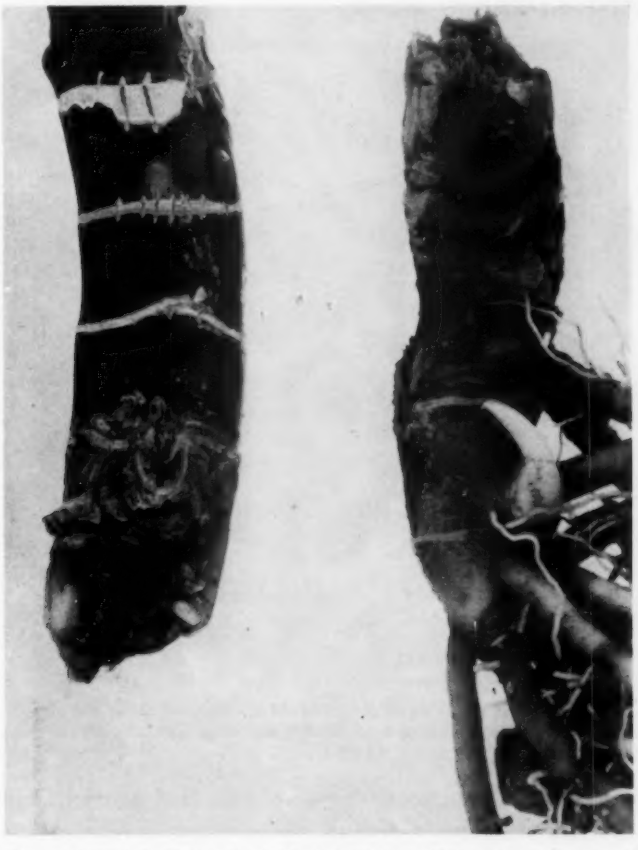

FIG. 2. Stem of an infected *Dieffenbachia picta* plant, grown from a stem or cane cutting, immediately after lodging.

to young plants, the foliage of older, infected plants does not wilt, either before or after the main stem collapses. The leaves and petioles retain their normal turgidity and color, probably because of the rapid spread of the disease in a relatively restricted area and the high water content of the succulent main stem.

Internally, as viewed in longitudinal section, the invaded tissues are water-soaked to dark gray, and frequently a definite, narrow, black band separates the diseased from healthy tissues (Fig. 3).

Apparently the roots of diseased plants are not affected (Fig. 2).

The disease is favored by relatively high air temperatures, high humidity, poor soil drainage, excessive irrigation, and crowding of the potted plants on greenhouse benches.

THE CAUSAL FUNGUS, PHYTOPHTHORA PALMIVORA

Numerous tissue plantings from naturally-infected stems of two commercial varieties of *Dieffenbachia picta* were made on malt-extract agar and have consistently yielded a fungus in pure culture which has been identified as *Phytophthora palmivora* Butler.

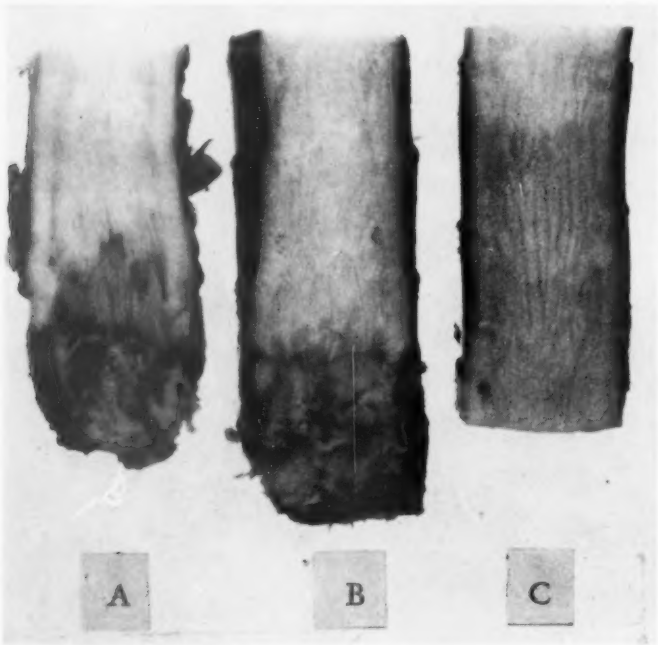

FIG. 3. Longitudinal sections of stems of *Dieffenbachia picta;* A, B. Diseased stems, naturally infected, in advanced stage of decay, showing narrow, black margin adjoining healthy tissues; C. Healthy stem.

Three isolates of the fungus which were studied proved identical. Sporangia and chlamydospores developed abundantly in oatmeal agar cultures, and on tufts of mycelium washed and transferred from pea broth to sterile distilled water.

The sporangia are produced sympodially on slender sporangiophores not inflated or swollen at the nodes and differing but little from the vegetative hyphae. The sporangia are limoniform, with a rounded base, and supported by a pedicel which, on abscission, often remains attached to the sporangium. At the apex the sporangium is provided with a broad, prominent, convex, hyaline papilla. Mature sporangia germinate by one or numerous germ tubes, usually arising adjacent to the papilla, or by the

development of zoospores of the usual Phytophthora type, completely differentiated within the sporangium, and escaping through the orifice resulting from the dissolution or rupture of the papilla. The size of the sporangia varies from 26 to 80 microns in length and 18 to 36 microns in diameter, with a mean size of 48.4×27.8 microns.

Chlamydospores are terminal or intercalary, spheroidal, hyaline at first but becoming straw color to brown in older cultures. Considerable thickening of the wall often occurs. Germination is always by germ tubes. The diameter of the chlamydospores ranges from 21 to 51 microns, with a mean of 32.7 microns.

Oogonia and oospores were not observed.

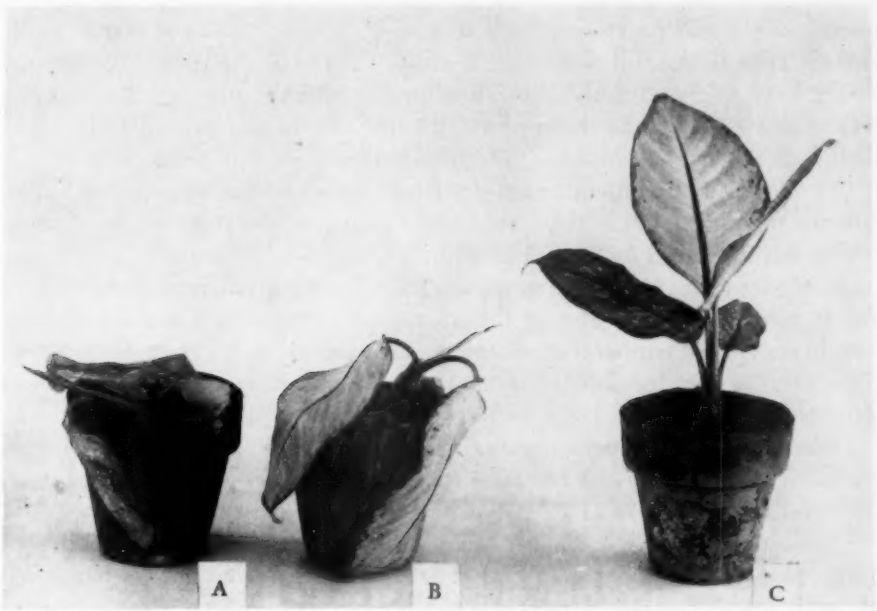

FIG. 4. *Dieffenbachia picta* plants, variety Rudolph Roehrs, grown from stem or cane cuttings. A, B. Diseased plants, showing advanced symptoms, 12 days after inoculation with *Phytophthora palmivora;* C. Healthy control plant.

The fungus corresponds closely to the description of *Phytophthora palmivora* by Butler (3) although he mistakenly regarded the chlamydospores as oogonia. Ashby (1) in 1929 emended the description and established the heterothallic character of the species. He also distinguished isolates of the species as "typical" or "atypical," basing the distinction principally on the amounts of sporulation in culture. The isolates from *Dieffenbachia* may be referred to his "typical" group, producing sporangia and chlamydospores early and profusely.

Isolates of the fungus proved pathogenic to healthy plants of the regular or dark-green, variegated leaf variety of *Dieffenbachia picta* and of the light-green, variegated leaf variety known as Rudolph Roehrs. Inoculum was pre-

pared by growing the fungus on poured plates of malt-extract agar. After 5 days' growth at room temperature, the agar inoculum was cut into ½-inch blocks, the depth of which averaged ⅛ inch. It was added to 6-inch pots of autoclaved soil, each containing a healthy plant, by making a small hole about ½ inch deep at a distance of 1 inch from the main stem, adding 1 or 2 blocks of inoculum, and covering with soil. All pots were heavily watered each day to keep the soil very moist, thus providing optimum conditions for infection. Greenhouse temperatures ranged from 70° to 80° F. while the inoculation tests were in progress. The incubation period ranged from 10 to 15 days for each variety of *Dieffenbachia,* and all infected plants died within 2 or 3 days after the stem and foliage collapsed (Fig. 4). Of 15 plants inoculated, of each variety, all became infected, while the 20 control plants, which had previously been treated by adding blocks of sterile malt-extract agar to the soil, remained healthy. Infected plants had symptoms which were identical with those of naturally-infected plants. The fungus was reisolated from the stems of all infected plants and proved to be identical with the original isolate. The reisolates were highly pathogenic.

The relation of temperature to growth of the mycelium was studied. The culture tubes (2.1 by 20 cm.) used and the procedure followed were those previously described by Tompkins and Gardner (5). The medium used was malt-extract agar, pH 7.0. Inoculated tubes were kept at room temperature for 48 hours. Then 3 tubes of the isolate were placed in a horizontal position in controlled temperature chambers at intervals of 3°, from 4° to 40° C. The cultures were incubated for 96 hours. The cardinal temperatures were determined on the extent of mycelial growth in the culture tubes.

The minimum temperature for growth of the isolate of *Phytophthora palmivora* from *Dieffenbachia picta* was approximately 13° C., the optimum 28°, and the maximum 31°.

Tucker (7), using cornmeal agar with pH 6.2, found that isolates of *Phytophthora palmivora* grew rather profusely at 30° or 32.5° C., but failed to develop at 35°. Of 42 isolates, 4 made no growth at 32.5°, while 38 grew at this temperature, many of them profusely. However, none proved capable of appreciable growth at 35°. Typical and atypical isolates behaved very similarly. The fungus developed most abundantly at 27° to 30°. At the lower temperatures, 1 isolate grew at 5°, 25 at 10°, 15 at 15°, and 2 at 20°. Isolates of the fungus grew most frequently at 10°.

EXPERIMENTAL HOST RANGE

Phytophthora palmivora is a tropical species, characterized by a wide host range. No attempt has been made to review the literature which is scattered and voluminous.

Among species of the Araceae Phytophthoras have been reported on the dasheen (*Caladium Colocasia* (L.) Wight.) and on the calla (*Zantedeschia sp.*). *P. colocasiae* Rac. was described by Raciborski (4) from the dasheen in Java in 1900 and has been identified in numerous Asiatic and Pacific areas.

It is unknown in the Americas. Infection is usually confined to the leaves where the fungus causes the development of brown spots. However, under favorable conditions invasion of petioles and corms may occur. On the calla (*Z. aethiopica* Spreng.) *P. richardiae* Buis. was described in 1927 by Miss Buisman (2) in Holland as the cause of a root rot. The disease, or one with similar symptoms, has been observed in England and at various locations in the United States. In 1947 Tompkins and Tucker (6) described a leaf blight of the pink calla (*Z. rehmannii* Engler) caused by *P. erythroseptica* Pethyb. The disease is known only in California.

Studies on the host range of *Phytophthora palmivora* from *Dieffenbachia* in the greenhouse have been limited to those plants grown most commonly in the same environment in local greenhouses for the florists' trade. Employing the same inoculation technique as heretofore described, 8 plants each of *Schismatoglottis latifolia* Miq., *Aglaonema simplex* Blume, *A. commutatum* Schott, *Philodendron cordatum* (Vell.) Kunth, *Caladium bicolor* Vent, tuberous-rooted begonia seedlings (*Begonia tuberhybrida* Voss), Gloxinia (*Sinningia speciosa* Benth. & Hook.), and pink calla (*Zantedeschia rehmannii* Engler) were tested. No infection was obtained, indicating that the disease under local conditions is confined to the 2 varieties of *Dieffenbachia picta* previously mentioned.

CONTROL OF THE DISEASE

Experimental tests conducted in a greenhouse in San Francisco, California, indicate that this disease can be avoided by rooting stem or cane cuttings in 2-inch pots of steam-sterilized sand. After the formation of a good root system, requiring from 4 to 5 months, the young plants were transferred to 6-inch pots of steam-sterilized soil in which they continued healthy as long as they were held prior to sale. Cuttings rooted in non-sterile sand and grown in soil known to be naturally infested with the organism continued to show a high percentage of diseased plants.

Additional protection against the disease may be provided by dusting the cuttings with a suitable fungicide. In tests which were also conducted in San Francisco, 55 cuttings were dusted with each of the following fungicides: Spergon (mixed with Celite 505 in equal parts by volume), Fermate (mixed with Celite 505), Arasan, Phygon, and Zerlate. Controls consisted of 55 cuttings dusted with Celite 505 and 55 untreated cuttings. All cuttings were then placed in 2-inch pots of steam-sterilized sand on a greenhouse bench. After 4 months, examination showed that the cuttings dusted with Fermate (ferric dimethyldithiocarbamate), Phygon (2,3,dichlor-1,4-naphthoquinone), and Spergon (tetrachloro-parabenzoquinone) had developed excellent root systems and vigorous, healthy foliage. Less promising results were obtained with Arasan (tetramethyl-thiuram-disulfide) and Zerlate (zinc dimethyldithiocarbamate), while the controls yielded the poorest results. Apparently the use of certain fungicides as surface dusts on cuttings of *Dieffenbachia picta* affords protection against infection and stimulates

root and foliage growth. Following the transfer of the rooted cuttings to 6-inch pots of steam-sterilized soil, stem rot has not occurred on any of the plants during the succeeding months.

Well-drained soil and careful watering of the plants in the greenhouse are factors which contribute toward freedom from disease.

SUMMARY

Stem rot of two varieties of *Dieffenbachia picta* occurs in commercial greenhouses in San Francisco, California.

Symptoms of the disease consist of a rapid, wet decay of the stem at the soil level. The foliage of young plants turns yellow and wilts, the top falls, and the plant dies. The foliage of older plants does not wilt but remains turgid and green, both before and after the main stem lodges.

The causal organism has been identified as *Phytophthora palmivora* Butler, on the basis of morphologic characters and temperature-growth relations.

In the greenhouse, infection was obtained by adding the fungus on agar blocks to pots of steam-sterilized soil containing healthy plants grown from cane cuttings. The incubation period averaged 12½ days.

The isolate of the fungus from *Dieffenbachia picta* failed to infect certain ornamentals tested in a comparable environment.

The minimum temperature for mycelial growth was 13° C., the optimum 28°, and the maximum 31°.

The disease is favored by high air temperatures, high humidity, poor soil drainage, excessive irrigation, and crowding of the potted plants in the greenhouse.

The disease can be avoided by rooting cane cuttings, previously dusted with Fermate, Phygon, or Spergon, in small pots of steam-sterilized sand. Subsequently, in shifting the rooted cuttings to larger pots, steam-sterilized soil should be used. Care in watering the plants and good drainage are essential.

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NITROGEN, POTASSIUM, AND CALCIUM IN RELATION TO FUSARIUM WILT OF MUSKMELON[1]

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INTRODUCTION

Fusarium wilt of muskmelon (*Cucumis melo* L.) caused by *Fusarium bulbigenum* (Cke. and Mass.) var. *niveum* Wr. f. 2 has been fully described in Minnesota by Leach and Currence (6). The disease now occurs in a number of muskmelon producing areas in the United States including Maryland where it is serious in Anne Arundel County.

The relation of nutrition to disease resistance has been the subject of extensive study, and there have been numerous instances in the literature in which plant diseases have been partially controlled by the modification of fertilizer practices. A comprehensive review of this subject has been written by Wingard (17). Of particular interest are those papers pertaing to the relation of nutrition to the control of diseases caused by species of *Fusarium*.

Fisher (4), in his study of Fusarium wilt of tomato, found that a heavy application of lime plus low nitrogen reduced the number of plants invaded by the pathogen. Cook (2), working with the same disease, obtained similar results when a low nitrogen nutrient was supplied. Thomas and Mack (13), using the foliar diagnosis technique, analyzed tomato plants grown on wilt-infested soil. Sampling before symptoms of the disease appeared, they found that those plants which remained healthy contained more potassium than did those plants which subsequently became diseased. They found, also, that plants which became diseased had a low calcium content.

Sherwood (11) observed that the highest percentage of tomato wilt always occurred in the most acid soils in his experiments. Scott (10) noted that less Fusarium wilt of tomato occurred when the plants were grown at a pH of 6.4 to 7.0 and that more wilt appeared under more acid or more alkaline conditions.

Neal (8), Smith (12), Tisdale and Dick (14), and Young and Tharp (18) have reported that the application of high potash fertilizers, particularly to soils deficient in potassium, reduced the severity of wilt in cotton caused by *Fusarium vasinfectum* Atk. The effect of various nitrogen levels alone was not significant (8, 14) although Young and Tharp (18) felt that the application of sufficient amounts of potash to balance the available nitrogen and phosphorus was highly important in controlling the cotton wilt disease.

[1] Scientific Paper No. A168, Contribution No. 2065, Department of Botany, University of Maryland, Agricultural Experiment Station.

Walker and Hooker (15), in their study of cabbage yellows caused by *Fusarium conglutinans* Woll., found that, when plants were grown in sand culture, reduction of the potassium level consistently increased the severity of the disease.

The present study was undertaken to determine whether nitrogen, potassium, and calcium nutrition influences the susceptibility of muskmelon to Fusarium wilt.

MATERIALS AND METHODS

Inoculum and Inoculation Technique. The highly pathogenic culture of *Fusarium bulbigenum* var. *niveum* f. 2 which was used throughout the greenhouse and laboratory experiments was the third successive single spore isolate from a culture isolated from a wilted muskmelon plant. In the field experiment, an area was selected which was naturally infested with the pathogen.

Inoculum was prepared by growing the fungus on liquid Leonian's medium[2] in Erlenmeyer flasks. When the fungus mat had covered the surface of the medium (approximately 15 days at 27° C.), the cultures were filtered, and the mat was ground for one minute with tap water in a Waring Blendor. The suspension of spores and mycelial fragments was used without further treatment. Plants were inoculated by making a dibble hole in the sand adjacent to the tap root and pouring 15 ml. of inoculum on the roots thus exposed.

Culture of Plants in Greenhouse. All greenhouse experiments were planned as randomized block designs with at least four replications in each block. Seeds of the variety Bender's Surprise treated with tetra-chloro parabenzo-quinone (Spergon) were sown directly into 2-gallon crocks of steam-sterilized, quartz sand. In early experiments fine sand (predominately of 40-mesh grade) was used. When it was shown (9) that a coarse sand (predominately of 20-mesh grade) was superior for muskmelon culture, this grade was used in all subsequent experiments. The application of nutrient solutions was begun after the first true leaves appeared.

Nutrient solutions in tap water were prepared daily from molar stock solutions of the following salts: $Ca(NO_3)_2 \cdot 4H_2O$, KH_2PO_4, $MgSO_4 \cdot 7H_2O$, $CaCl_2O$, KCl, KNO_3, $Mg(NO_3)_2 \cdot 6H_2O$, $NaNO_3$. Throughout the study the following elements were supplied at constant rates as follows: calcium at 240 p.p.m., magnesium at 48 p.p.m., and phosphate at 190 p.p.m. The solutions were supplemented by the addition of measured small amounts of manganese, zinc, copper, boron, and iron. Nutrient solutions were applied to the crocks at the rate of 500 ml. once a day. Crocks were thoroughly flushed with tap water once a week.

Culture of Plants in the Field. The field experiment was conducted on a farm in Anne Arundel County. Three years before its use in this experiment, the field used was planted with muskmelons and, according to the

[2] Leonian's medium: potassium monobasic phosphate 1.20 gm., magnesium sulphate 0.60 gm., peptone 0.60 gm., maltose 6.25 gm., malt extract 6.25 gm., and water 1000 ml.

owner, the crop had been a total loss because of Fusarium wilt. After the crop failure, the field lay fallow for one year and then was planted with vetch. This cover crop was turned under in March, and in mid-June the land was prepared for muskmelons at which time soil samples were taken. Analysis[3] showed the soil to be a sandy loam with a pH value of 5.1 and containing 0.4 per cent organic matter. Its general fertility level was low.

The design of this experiment was that of a split block with lime vs. no lime as the whole plot. Whole plots were replicated 5 times. Hydrated lime was applied broadcast prior to the final harrowing at the rate of 1500 lb. per acre. This amount was calculated to raise the soil pH to 6.0. Fertilizer formulas were applied in the bottom of the rows at the rate of 800 lb. per acre. These formulas were 6–6–5 (normally used for muskmelons by the majority of farmers in the area), 4–8–8, 4–8–12, 4–8–16, and 4–8–20.

TABLE 1.—*System used for rating muskmelon plants infected with Fusarium wilt*

Rating	Description
0–4	Healthy or with slight to moderate discoloration of vascular system at soil line.
5–9	One branch root necrotic and with reddish-brown discoloration extending as an external streak up the tap root. Several drops of reddish-brown exudate on stem at first or second internode. Tip leaves of one runner slightly yellowed and cupped or runner nearly dead. Rest of plant normal.
10–14	One branch root necrotic and with reddish-brown discoloration extending as an external streak up the tap root. Brown discolored streak on approximately half of main stem. Streak may also extend part way along branch stem. Leaves more or less yellowed and cupped on stems with a streak. One or more branch stems normal.
15–19	One or more branch roots necrotic and with reddish-brown streak extending externally up the tap root. Brown streak on main stem and on all branch stems except one. Leaves more or less yellowed and cupped on stems with streak. One or more branches dead.
20	Entire plant dead.

Muskmelon plants, Clark variety, were propagated from seed in No. 2 tin cans filled with a compost-sand mixture in a cold frame. When two true leaves had appeared they were transplanted to the field. All plants received the same fertilizer in the cans that they later received in the field. Fifty-four days after transplanting, all plants were examined and rated for Fusarium wilt.

Disease Criteria. In the first greenhouse experiments the plants were rated as either dead or healthy. In subsequent experiments plants were rated according to a system developed for the field experiment. A description of the classes in this system appears in table 1 and diagrams of plants in some of the classes are shown in figure 1. Within each classification, consideration was given to the total amount of the plant affected including the extent to which fruit-bearing was prevented. Thus, a diseased plant which matured a marketable fruit on an apparently healthy runner received

[3] Analysis made by the Soils Department, University of Maryland.

a slightly lower rating than a similarly diseased plant which matured no fruit. In the last greenhouse experiment the plants were cut at the ground line, and freehand sections were made one inch below the cotyledonary node. Disease ratings were made on the basis of the percentage of the cross section of the stem invaded by the fungus hyphae.

Nutrition Experiments on the Pathogen. Spore suspensions of the pathogen were transferred to flasks containing 25 ml. of autoclaved nutrient

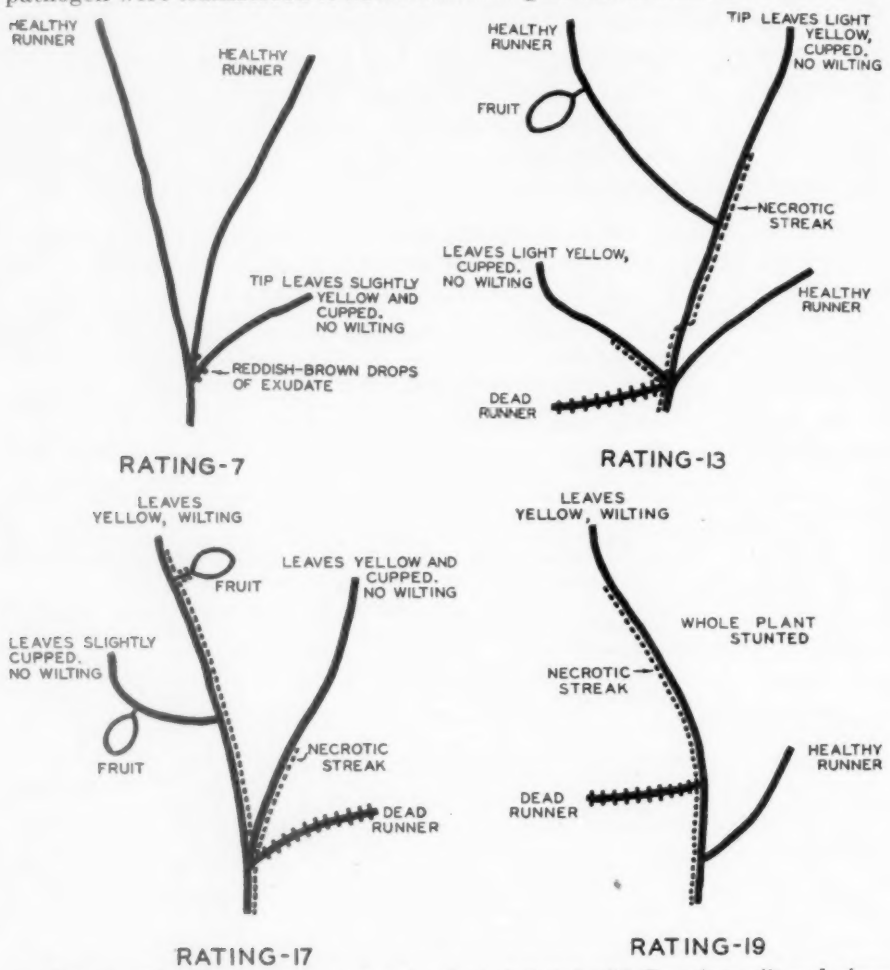

FIG. 1. Diagrams of four muskmelon plants infected with Fusarium wilt and given disease ratings as indicated.

solutions identical with those supplied to the muskmelon plants. After ten days' growth at 27° C., the mats were filtered off, oven dried, and weighed. In a second experiment the same procedure was followed except that nutrient solutions were divided into three pH series; pH 3.3, pH 6.0, and pH 8.0. The three series were buffered and adjusted to their respective pH levels. Representative flasks were titrated every three days and the pH of all flasks adjusted.

RESULTS

Greenhouse Experiments. Preliminary tests were run to determine the nutrient balance necessary for optimal growth of muskmelon plants in quartz sand and to ascertain the differences in nitrate content of main stems of plants grown on varying levels of nitrogen and potassium. The results showed that no significant increase in growth occurred after a level of 447 p.p.m. of nitrate was reached and that variations in the potassium level had no significant effect on total growth, although plants receiving 117 and 156 p.p.m. of potassium made more growth than did those receiving 78 and 195 p.p.m. The nitrate content of the stems, as analyzed by the rapid method of Carolus (1), had a high correlation with that supplied in the nutrient solution. At the lowest level of potassium (78 p.p.m.) there was significantly more nitrate than at any of the higher levels (117, 156, and 195 p.p.m.). At 447 p.p.m. of nitrate and above, regardless of the level of po-

TABLE 2.—*Correlations between the amount of nitrate and the amount of potassium in the nutrient solution and the number of muskmelon plants killed by Fusarium wilt*

P.p.m. nitrate	Plants killed[a]	P.p.m. potassium	Plants killed[b]
100	10	78	28
275	12	117	24
447	17	156	19
620	25	195	19
744	26	.....	.....
$r = 0.977^{**}$		$r = -0.947^{*}$	

** Significant at the 1 per cent point.
* Significant at the 5 per cent point.
[a] Total of 32 plants in each class.
[b] Total of 40 plants in each class.

tassium, the plants had a healthy green appearance. At concentrations of nitrate below 447 p.p.m., plants were a light green and resembled plants with symptoms of nitrogen deficiency.

Since, in the first inoculation experiment, results were recorded simply as plants killed by the pathogen, correlations on the relation between the number of plants killed and the amount of nitrate and the amount of potassium supplied were calculated. The results (Table 2) show that there was a highly significant correlation between mortality and the amount of nitrate supplied. The correlation between mortality and the potassium supply was significant at the 5 per cent point.

In the next experiment the plants were removed from the sand thirty days after inoculation and rated according to the system developed for the field experiment (Fig. 1 and Table 1). The results (Table 3) show a highly significant difference between the 100 p.p.m. and the 447, 744, and 992 p.p.m. nitrate levels but no significant difference between potassium levels or for the interaction between nitrate and potassium.

Fusarium was reisolated from every plant in the experiment irrespective of disease rating. The 160 isolates obtained were classified into eight

TABLE 3.—*Effect of inoculation with Fusarium wilt organism on muskmelon plants grown in quartz sand under various levels of nitrate and potassium*

P.p.m. nitrate	P.p.m. potassium				Mean disease rating per plant
	78	195	351	507	
100	0.7[a]	6.0	8.0	8.5	5.8
447	11.5	9.7	11.8	12.7	11.4
744	13.6	13.2	7.7	12.2	11.7
992	11.2	10.0	8.0	14.0	10.8
Mean disease rating per plant	9.3	9.7	8.9	11.9	

L.S.D. at 1 per cent point between means—4.2
L.S.D. at 5 per cent point between means—2.2

[a] Basic figures represent average rating for 10 plants.

different groups according to gross morphological differences and a representative from each was tested for pathogenicity on young muskmelon seedlings. All groups were pathogenic except one consisting of two isolates.

Since it has been shown in the previous experiment that even apparently healthy plants were invaded by the Fusarium wilt fungus, an experiment was set up to determine the extent to which plants would be invaded when grown under different nutritional levels. The data (Table 4) show a highly significant difference between the 100 p.p.m. level of nitrate and the 447 and 744 p.p.m. levels. There was no significance among any of the potassium levels or in the nitrate-potassium interaction.

Field Experiment. Preparatory to this experiment, all of Anne Arundel County was surveyed to determine the extent of the disease and the cultural procedures commonly used in the production of muskmelons. The fertilizer ratio generally used by the growers was 6–6–5. On the majority of farms, lime was seldom used and most of the soil tested was in the pH range of 4.5 to 5.5. In one field visited, the apparent result of soil acidity was striking. This field was divided into three definite sections: (1) all plants healthy, soil pH of 5.5, (2) 95 per cent of the plants dead from Fusarium

TABLE 4.—*Cumulative percentage invasion by the Fusarium wilt fungus of the hypocotyl of plants grown on various levels of nitrate and potassium*

P.p.m. nitrate	P.p.m. potassium			Total percentage[a] invasion
	78	156	273	
100	23	44	116	183
447	139	843	725	1707
744	731	723	858	2312
Total percentage[a] invasion	893	1610	1699	

L.S.D. at 1 per cent point between totals—1468
L.S.D. at 5 per cent point between totals—1074

[a] Represents total of 54 plants.

wilt, soil pH 4.1, and (3) 50 per cent of the plants dead or severely affected by Fusarium wilt, soil pH 4.8. In many other fields visited, calcium deficiency symptoms were noted. Because of these observations, it was decided to include in the field experiment lime plots in which the pH would be raised to 6.0, a level consistent with that recommended for muskmelon culture (5, 16).

The data obtained (Table 5) showed highly significant differences between the limed and unlimed plots. There were no interaction effects between lime and any of the fertilizer ratios used. The difference beween the check fertilizer, 6–6–5, and the other ratios was highly significant: there was no significance within the 4 per cent nitrogen series except that the 4–8–20 ratio was significantly better, at the 5 per cent point, than the 4–8–8 and 4–8–16 ratios.

TABLE 5.—*Amount of Fusarium wilt in muskmelon plants grown in wilt-infested soil modified by lime and various fertilizer ratios*

	Fertilizer ratio					Mean disease rating per plot
	6–6–5	4–8–8	4–8–12	4–8–16	4–8–20	
Lime	5.9[a]	4.2	3.4	3.4	3.0	3.95
No lime	8.8	5.2	4.9	5.9	3.3	5.63
Mean disease rating per plot	7.32	4.69	4.15	4.65	3.15	.....

L.S.D. at 1 per cent point between means for lime—0.83
L.S.D. at 1 per cent point between means for fertilizers—1.65
L.S.D. at 5 per cent point between means for fertilizers—1.25

[a] Basic figures in table are averages of 5 plots.

Effect of Nutrient Solutions on Growth of the Pathogen in Vitro. In a preliminary experiment the pH of the nutrient solutions was not controlled. The results showed a high correlation between the total growth of the pathogen and the amount of nitrogen in the medium. Levels of potassium of 351 and 429 p.p.m. produced significantly less growth than did the 273 p.p.m. level.

In the experiment where the pH of the nutrient solutions was controlled the fungus tended to raise the pH from pH 3.3 and lower the pH from pH 6.0 and 8.0. The total weights in milligrams of the *Fusarium* were 1244 mg. at pH 3.3, 274 mg. at pH 6.0, and 585 mg. at pH 8.0. The L.S.D. at the 1 per cent point for these weights was calculated to be 124.4 mg. Thus the differences among all three pH levels was highly significant. The complete analysis of variance for this experiment appears in table 6. It should be noted that the variance for pH levels is very large. In order to evaluate more fully the effects of pH on the growth of the fungus, the figures within each pH series were analyzed separately. The data are presented in table 7. At pH 3.3 there was a highly significant increase in growth of the fungus with each increase in nitrate level. At pH 6.0 no significant increase in

TABLE 6.—*Analysis of variance of experiment in which the Fusarium wilt organism was grown for ten days under various levels of nitrate and potassium within each of three pH series*

Source of variation	Degrees of freedom	Variance	"F" value
Total	107		
Nitrate	2	1714.75	52.940**
Potassium	2	5.50	0.190
pH	2	6814.35	210.384**
$NO_3 \times K$	4	52.62	1.613
$NO_3 \times pH$	4	164.02	5.064**
$K \times pH$	4	129.07	3.985**
$NO_3 \times K \times pH$	8	241.24	7.448**
Error	81	32.39	

** Significant at the 1 per cent point.

growth occurred until the highest level of nitrate was attained, whereas at pH 8.0 there were no significant differences in total growth between any of the nitrate levels. When pH was held at 3.3, solutions containing 78 p.p.m. of potassium produced significantly (at 5 per cent point) more growth than did solutions containing 195 p.p.m. of potassium. When the fungus was grown at pH 6.0 and 8.0, potassium levels had no effect on total growth.

TABLE 7.—*Growth in milligrams (dry weight) of Fusarium bulbigenum var. niveum f. 2 in nutrient solutions containing three levels of nitrate and potassium and held at three pH levels for ten days*

pH	P.p.m. nitrate	P.p.m. potassium			Mean
		78	156	195	
3.3	100	24.8[a]	25.8	19.5	23.3
	273	40.0	39.8	30.5	36.7
	447	46.3	41.8	42.8	43.6
	Mean	37.0	35.7	30.9	
	L.S.D. at 1 per cent point between means—6.8 L.S.D. at 5 per cent point between means—5.0				
6.0	100	−1.3[a]	2.3	4.3	1.8
	273	2.5	2.8	9.3	4.8
	447	14.0	23.5	11.3	16.3
	Mean	5.1	9.5	8.3	
	L.S.D. at 1 per cent point between means—9.2 L.S.D. at 5 per cent point between means—6.8				
8.0	100	14.3[a]	6.5	19.8	13.5
	273	18.5	15.3	18.3	17.3
	447	19.5	18.3	16.0	17.9
	Mean	17.4	13.3	18.0	
	Differences between means not significant.				

[a] Basic figures represent average weights of 4 replications.

DISCUSSION

Throughout the course of this study, a relationship between nitrogen nutrition and the behavior of *Fusarium bulbigenum* var. *niveum* f. 2 has been observed. In the greenhouse inoculation experiments an increase of nitrogen in the nutrient solution resulted in a significantly greater invasion of the plant by the fungus and a larger number of the plants killed. There is little evidence that potassium plays an important part in affecting susceptibility when nitrogen and potassium are balanced and in amounts sufficient for normal growth of the muskmelon plant. In the field experiment the highly significant reduction in the amount of disease occurred between the 6–6–5 and the 4–8–8, 4–8–12, 4–8–16, and 4–8–20 fertilizer ratios. Within the 4 per cent nitrogen ratios there were no highly significant differences. It is possible that these results can be attributed more to the reduction of the percentage of nitrogen rather than to an increased percentage of potassium.

The nutrition studies of the *Fusarium, in vitro,* indicate that nitrogen is the important element governing the growth of the fungus. Cox (3), in his work on the host-parasite relationship in muskmelon wilt, found that in the early stages of the disease the fungus enters the roots and primarily inhabits the xylem although other tissues are invaded later. It appears, then, that the *Fusarium* must depend largely, at least in the early stages of infection, on elements in the xylem stream for its nutrition. It is possible that an abundance of soluble nitrogen in the xylem could influence the extent of invasion. The possibility that an abundance of nitrogen makes host cells more susceptible to invasion by the parasite cannot be overlooked.

The effects of pH on the amount of disease present in the field were similar to those reported by Sherwood (11) and Scott (12) who worked with Fusarium wilt of tomato. The field results with lime also parallel the data obtained in the laboratory where the fungus, *in vitro,* made the poorest growth when held at a pH of 6.0. Since there was no significant interaction between lime and any of the fertilizer ratios, it is possible that the lowered disease index in the limed plots resulted from inhibiting the growth of the fungus in the soil or in lowering its virulence. It is recognized, however, that muskmelons grow best when the soil reaction is slightly acid or neutral (5, 16). That muskmelons have a high calcium requirement is also well known (7). Some of the beneficial effects of lime, therefore, may have come from providing the plants with soil conditions that were more nearly optimum.

SUMMARY

The observations obtained from greenhouse and field experiments indicate that high levels of nitrogen result in a significantly greater invasion of muskmelon plants by *Fusarium bulbigenum* var. *niveum* f. 2 and a larger number of plants killed. There was little evidence that potassium

plays an important part in affecting susceptibility when nitrogen and potassium are balanced and in amounts sufficient for normal growth of the muskmelon plant. *In vitro*, the pathogen made more total growth as the nitrogen content of the medium was increased; however, little correlation was observed between total growth and potassium supply.

The addition of lime to the soil in an amount sufficient to raise the soil pH to 6.0 significantly reduced the amount of disease in the field plots. Part of the reduction may be attributed to the change in pH since, *in vitro*, the growth of the pathogen was drastically inhibited when the pH of the medium was held at 6.0.

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A PHYSODERMA DISEASE OF QUACK GRASS

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While searching for the stripe smut *Ustilago striiformis* (West) Niessl on various grasses in the fields about Madison, Wisconsin, an apparently new disease was found on *Agropyron repens* (L.) Beauv. A cursory study revealed that the disease was incited by a species of *Physoderma,* new for Wisconsin and perhaps for the United States.

The symptoms of the disease are distinctive. Internodal elongation of the culms is reduced and the leaf blades develop erectly. The young leaves are light green and the older leaves have small pale yellow striae that gradually change to rusty brown stripes as the numerous striae coalesce (Fig. 1, B). In external appearance the symptoms simulate those of downy mildew on the grasses. The mode of development suggests a systemic type of infection although the resting sporangia are produced in the leaf blades and sheaths. Basal rotting of the culm and wilting and drying out of the leaves occurs in the case of the more severe infection (Fig. 1, A). No infection of the root cortical tissues has been evident in the field material.

Sections through the infected leaves show numerous resting sporangia of a species of *Physoderma.* In the initial stages of fungus development in the young tissues, the delicate rhizomycelium is evident in the host cells. The sporangia form, filling the parenchymatous cells, and resemble in essential features those of *Physoderma zeae-maydis* Shaw on corn. Mature sporangia are reddish-brown, thick-walled, smooth, 20–40 μ in diameter with granular cell contents. The spores are slightly flattened on one side indicating the position of the operculum (Fig. 2).

Sporangia scraped out of mature sori were placed in small quantities of water on slides and inclosed in moist chambers at 24°–30° C. Germination of a few sporangia that were observed was similar to that described by Tisdale (5) for *Physoderma zeae-maydis.* The operculum is pushed aside by the protrusion of the endospore and the contents of the sporangium form numerous zoospores. Further observations are being made on the behavior of the zoospores and the mode of infection.

The fungus under study closely resembles *Physoderma zeae-maydis* on corn, but differs slightly in the type of symptoms produced as well as in the measurement of the sporangia. In *P. zeae-maydis,* the infection tends to be localized, whereas in the *Physoderma* under study, the infection is probably systemic. Furthermore, in the former the mature sori are erumpent, whereas in the latter, the sori are nonerumpent and the sporangia are released only by the decay of the leaf tissue. Sporangia of *P. zeae-maydis* measure 18–24 × 20–30 μ in comparison with 20–40 × 20–34 μ in the *Physoderma* on quack grass.

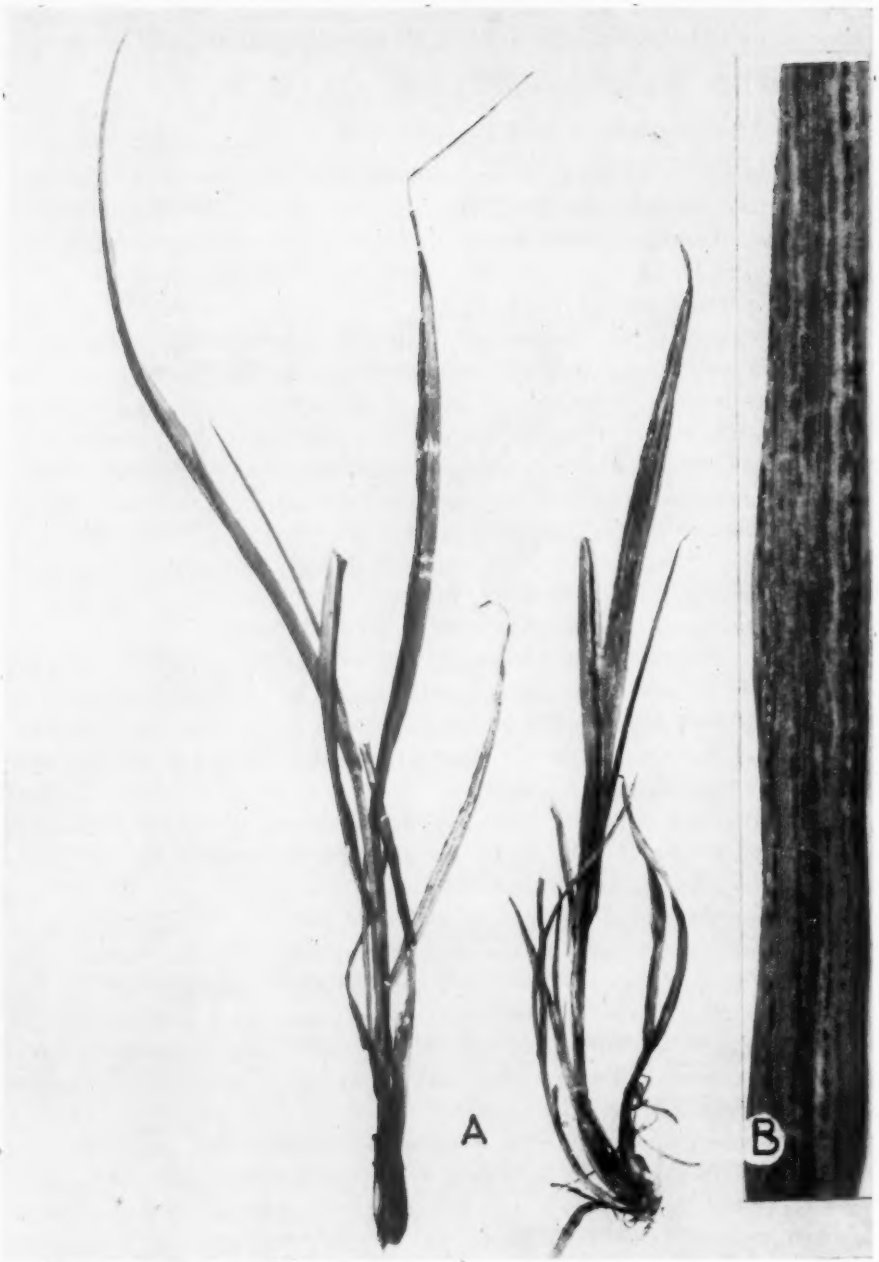

FIG. 1. Culms of *Agropyron repens* infected by *Physoderma*. A. Plants with stripes on the leaves, stunted growth, and rotting at the base of the culms. × ¾ nat. size. B. Enlarged leaf blade showing the numerous striae coalescing to form brown stripes.

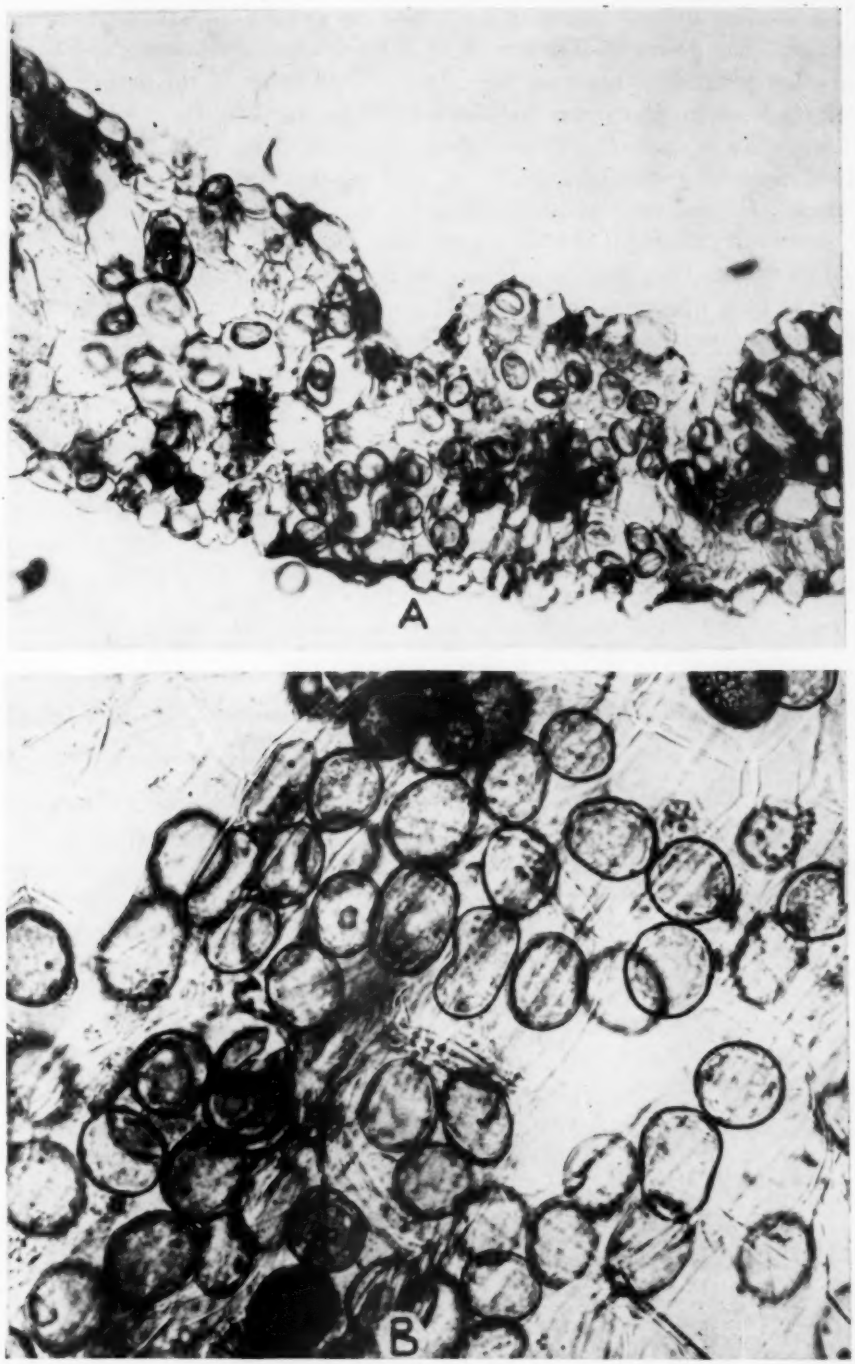

FIG. 2. Resting sporangia of the *Physoderma* on *Agropyron repens*. A. Transection through the diseased leaf showing the sporangia in the mesophyll cells. About 80×. B. Typical masses of the sporangia. About 300×.

A similar disease has been described in Europe on several economic grasses. Sampson and Western (3, p. 33) described a yellowing and damping-off of grass seedlings especially *Agrostis spp.* when in the first or second leaf stage as probably due to *Cladochytrium caespitis* Griff. and Maubl. This species is similar to *Physoderma* as discussed by Cook (1) who combined the two genera; Sparrow (4) and others, however, retain the two genera. *Physoderma agrostidis* Lagerh. on *Agrostis gigantea* Roth and *P. graminis* (Büsgen) Fischer on *Dactylis glomerata* L., *Agropyron* (*Triticum*) *repens* (L.) Beauv., *Alopecurus pratensis* L., and *Phleum pratense* L. have been described in Europe (2). The species found near Madison, Wisconsin closely resembles *P. graminis* in morphology; however, sporangia have not been found in the root cortex as described for this species. The disease was not found on the other three grasses although they were growing in close proximity to the infected quack grass. In so far as the authors can determine this is the first report of this fungus in North America.

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PHYSIOLOGIC RACES OF USTILAGO TRITICI IN THE EASTERN SOFT WHEAT REGION OF THE UNITED STATES[1]

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(Accepted for publication August 27, 1947)

INTRODUCTION

The first published description of physiologic races of *Ustilago tritici* (Pers.) Rostr. based on pathogenicity tests was by Piekenbrock (9) in 1927. Since that time other workers (3, 4, 5, 6, 7, 8, 11) have described a number of races of this fungus. In 1930 Grevel (4) working in Germany reported four races of loose smut of wheat. Radulescu (11) found no indication of physiologic specialization when he tested seven collections of loose smut from Romania on five standard winter wheat varieties. He did, however, find three races when he used twelve varieties of spring wheat as host differentials in testing eight collections of loose smut. In 1932 Hanna and Popp (6) working in Canada described two races of loose smut based on the reactions of common and durum wheats. Again in 1937 Hanna (5) described two additional races determined by tests on nine varieties of spring wheat, two durum varieties, one durum × spring wheat hybrid, and one emmer. In 1936 and in 1942 Moore (7, 8) reported five physiologic races that he had distinguished by reactions of certain bread and durum wheats, but did not name the varieties nor their reactions. These races were derived from twelve smut collections from Minnesota, North Dakota, Texas, and Mexico. Caldwell and Compton (3) reported host specialization among seven wheat loose smut collections from Illinois, Indiana, and Ohio. Working independently in Texas, Kansas, and Illinois on a study of wheat varietal reaction to loose smut, Atkins, Hansing, and Bever (1) found that the same variety might be susceptible at one location and not at another, which indicated that different physiologic races of the fungus were occurring in the different areas.

In 1940, as a part of the coordinated eastern soft winter wheat program, the writer started an intensive study on the problem of physiologic specialization in *Ustilago tritici*. This study involved pathogenicity tests of smut collections from all the wheat growing regions of the United States with special emphasis on smut collections from the eastern soft wheat region. The results here reported cover primarily smut from this latter region.

[1] Cooperative investigation of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the Illinois Agricultural Experiment Station.

[2] Pathologist, Division of Cereal Crops and Diseases. The writer is indebted to Mr. J. W. Taylor, Beltsville, Maryland, for supervision of the plantings and for smut data on the variety Leap used in this experiment and grown each year on the Plant Industry Station, Beltsville, Maryland. Thanks are also due Dr. A. G. Johnson for assistance in the preparation of the manuscript.

MATERIALS AND METHODS

The work was done at the Illinois Agricultural Experiment Station, Urbana, Illinois. All of the inoculations were made in the greenhouse.

The following winter wheat varieties were used as differentials: Wabash (C.I.[3] 11384), American Banner (C.I. 6943), Purdue No. 1 (C.I. 11380), Hussar (C.I. 4843), Early Premium (C.I. 11858), Nabob (C.I. 8869), Forward (C.I. 6691), Trumbull (C.I. 5657), Kanred-Gipsy (C.I. 11382), Leap (C.I. 4823), Kawvale (C.I. 8180), and Prairie (C.I. 12371). The results with Kawvale and Prairie are not included here because their reactions did not differentiate any of the smut collections thus far studied. Kawvale was resistant to all of them. Prairie was susceptible to most of them. The varieties were selected on the basis of physiologic race investigations by Caldwell and Compton and because of their possibilities as parent material in breeding for resistance to loose smut.

For the most part, the inoculum was collected prior to 1940 by B. B. Bayles, Division of Cereal Crops and Diseases, and each collection inoculated separately into the spring wheat variety Kota. The infected seed was given to the writer and the original inoculum employed in this study was taken from smutted plants grown from these various lots of infected Kota seed.

The smut inoculum for the first year's test was taken from the variety Kota. For the second year's test it was taken from two of the differential varieties and mixed, except that race 1 was taken from Wabash which was the only host differential infected. For the third and fourth year's test the inoculum was taken from the same variety each year.

In all, 52 smut collections from Texas, Missouri, Michigan and eastward have been classified into races on the basis of the reactions of the differential varieties grown in the field from infected seed produced by plants inoculated in the greenhouse.

The seed of the differential varieties to be inoculated was treated by the modified hot-water treatment to insure smut free plants. After the seed had dried for three or four days, it was treated with an excess of Semesan Jr. (one per cent ethyl mercury phosphate) as a precaution against contaminants during the vernalization process, which was as follows: From 300 to 400 kernels of the treated seed of each variety were put in a separate 155-mm. Petri dish with 20 cc. distilled water added and left at room temperature for 24 hours to start germination. The Petri dishes were then placed in a refrigerator at 2° to 4° C. and left 60 days, water being added as necessary. This period of vernalization was found optimum for most of the varieties. At the end of the 60 days the coleoptiles of most varieties were one-half to two and a half inches long and, in some varieties, the first leaf had pushed out of the coleoptile about one inch. The young seedlings were then planted in soil in 2-gallon glazed jars, 15–17 plants per jar in the greenhouse.

[3] C.I. refers to accession number of the Division of Cereal Crops and Diseases.

The soil in each jar was fertilized with 150 cc. nutrient solution[4] just before planting and again at each 10-day interval until the plants were in the early boot stage.

Two crops a year were grown in the greenhouse. For the fall crop, the the seed was vernalized during July and August and planted in early September; and the plants matured early in January. The spring crop was handled about four and a half months later.

The greenhouse temperature ranged from 50° to 95° F. However, as the season progressed a temperature of 60° to 70° F. was maintained until the plants were in the soft-dough stage when it was raised to 75° to 80° F. until the plants were mature.

During the short days of late fall and winter, daylight was supplemented by three 750-watt electric bulbs supplying 150 to 180 candle power, for each 2.5 × 17 foot bench, daily to March 15. By seeding in early September very little floral sterility was encountered.

At anthesis, which was approximately 60 days after the seedlings were planted in the jars, four heads of each of the differential varieties were inoculated with a malt-extract[5] suspension of chlamydospores of each smut collection. A spore dilution of 0.03 gm. spores per 100 cc. of malt extract was used. Other dilutions were tried but were less satisfactory. The method of inoculation described by Poehlman (10) was used except that a regular 20-cc. hypodermic syringe was employed instead of the rubber bulb, and malt extract was used instead of dextrose. The use of the hypodermic syringe and needle has several advantages, namely: a small amount of inoculum is required, adequate inoculum is assured for each floret, and there is little or no head mortality.

After the inoculated heads had matured, they were harvested and threshed individually. The seed from the 4 heads inoculated with the same smut collection was thoroughly mixed and divided into four groups and packeted. At fall seeding time each of the four lots of inoculated seed was sown in a separate hill in rows as described by Bonnett and Bever (2), 15 to 30 seeds in each hill and the hills 18 inches apart in rows two feet apart.

The infected seed of the winter-tender variety Leap was sown at the Plant Industry Station, Beltsville, Maryland, each year because the danger of winterkilling there is less than at Urbana, Illinois.

[4] The nutrient solution was made by mixing 125 cc. from each of the following stock solutions A, B, and C, with 18,900 cc. tap water.

Stock solution A	
Sodium nitrate ($NaNO_3$)	1012.05 gm.
Distilled water	7500.00 cc.
Stock solution B	
Sodium phosphate, monobasic, ($NaH_2PO_4 \cdot H_2O$)	100.12 gm.
Distilled water	7500.00 cc.
Stock solution C	
Potassium sulphate (K_2SO_4)	100.00 gm.
Distilled water	7500.00 cc.

[5] One per cent malt extract in tap water.

The percentage of smut was based on counts of the total number of heads in the four hills. The number of heads per hill ranged from 30 to 110, and thus there were from 120 to 440 heads for each variety each year.

After the first year the smut inoculum was secured from the same variety each year and stored in a refrigerator at 40° F. until used the following winter.

For clarity and simplicity, simple numbers are used to designate the races rather than pedigree numbers as employed by Hanna (5). Infection percentages are given in terms of the nearest whole number.

In the analysis of the data, two infection classes are considered. Any variety with 10 per cent smut infection or less is considered resistant. Varieties with 11 per cent or more are considered susceptible.

RESULTS

The average percentages of smutted heads of the differential varieties produced by the different races are given in table 1. The upper figure of each pair represents the average percentage infection from individual

TABLE 1.—*Average percentages*[a] *of smutted heads in 10 varieties of winter wheat inoculated with 11 physiologic races of Ustilago tritici*

Physiologic race no.	Average percentage smutted heads in									
	Wabash	American Banner	Purdue No. 1	Hussar	Early Premium	Nabob	Forward	Trumbull	Kanred-Gipsy	Leap
1	38	0	0	0	0	0	0	0	0	0
	45	0	0	0	0	0	0	0	0	0
2	46	20	18	14	0	0	14	0	0	0
	50	21	12	34	0	0	7	0	0	0
3	33	20	21	19	30	10	2	3	0	0
	43	24	25	29	32	17	0	0	0	0
4	40	43	44	19	26	25	10	0	0	0
	41	56	53	16	56	24	21	0	0	0
5	40	39	29	21	49	17	17	35	2	0
	38	40	16	10	35	20	10	32	0	0
6	42	33	28	14	42	15	22	42	24	7
	43	35	22	12	29	27	10	43	23	12
7	41	31	24	27	30	18	21	38	0	9
	44	55	12	12	56	11	17	40	0	21
8	58	0	43	29	0	0	48	39	0	0
	48	0	38	18	0	0	30	42	0	0
9	72	0	22	6	0	0	0	43	0	0
	48	0	20	0	0	2	1	50	0	0
10	56	0	0	0	0	0	0	1	37	0
	35	0	0	0	0	0	0	0	49	0
11	4	42	9	56	0	0	0	0	0	0
	0	51	0	40	0	0	0	0	0	0

[a] Upper figure of each pair represents the average percentage infection from individual collections during three years and the lower figure gives that obtained in one year from inoculum composited from collections that had given similar results for three years previously.

collections during three years and the lower figure of each pair gives the average percentage obtained in one year from inoculum composited from collections that had given similar percentages of smut for the three years previously.

In most cases each race is distinguished from the others by the reaction of more than one variety. The difference between races on the differentiating varieties usually was 20 per cent or more. The consistency of the results indicates that the smut inoculum was free from race mixtures.

No variety was susceptible to all of the races. While Wabash was susceptible to more races than any of the other varieties tested, it was resistant to race 11.

In certain years the plants in three hills of a variety smutted with a particular smut collection showed heavy infection, while the fourth hill had

TABLE 2.—*Distinguishing characteristics and original sources of 11 physiologic races of Ustilago tritici*

No.	Race	Original collections from–	
	Distinguishing characteristics	Variety	State
1	Wabash susceptible; all other differentials resistant.	Wabash, Purplestraw, Fultz, Fulcaster, Posey's Bluestem, Redrock	Ga., Ill., Ind., Ky., Mich., Mo., Tex.
2	Wabash, American Banner, Purdue No. 1, Hussar susceptible; others resistant.	Purplestraw, Honor, Forward, Clarkan	Ga., N. C., N. Y.
3	Differs from race 2 in that Early Premium and Nabob are susceptible and Forward is resistant.	Purplestraw, Fulcaster, Mediterranean, Grandprize	Ga., Ky., N. C., S. C., Tex.
4	Differs from race 3 in that Forward is susceptible.	Goens	Ky.
5	Differs from race 4 in that Trumbull is susceptible.	Purplestraw, Fultz	S. C., Ga., Tex.
6	Differs from race 5 in that Kanred-Gipsy is susceptible.	Purplestraw, Illinois No. 2, Early Premium	Ga., Md., N. C.
7	Differs from race 6 in that Kanred-Gipsy is resistant and from race 5 in that Leap is susceptible.	Trumbull, Fulcaster, Purplestraw	Ill., Ky., N. C., S. C., Tenn., Va.
8	Similar to race 2, but differs from it in that American Banner is resistant, Trumbull susceptible, and Forward susceptible.	Forward	N. C.
9	Similar to race 1, but differs in that Purdue No. 1 and Trumbull are susceptible.	Fultz	Tex.
10	Similar to race 1, but differs in that Kanred-Gipsy is susceptible.	Hope-Turkey, Fulcaster, Red May	Ark., Ind., Tex.
11	Similar to race 1, but differs in that Wabash is resistant and American Banner and Hussar are susceptible.	Turkey and two mixed varieties	Ill., Ohio, Tex.

TABLE 3.—*Number and distribution of races of Ustilago tritici in 52 collections from the eastern soft wheat area of United States*

State	Number of collections of races–											Total	Total number of races
	1	2	3	4	5	6	7	8	9	10	11		
New York	.....	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	1
Ohio	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	1	1
Indiana	1	.....	.....	.....	.....	.....	.....	.....	.....	2	.....	3	2
Illinois	1	.....	.....	.....	.....	.....	1	.....	.....	.....	1	3	3
Michigan	1	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	1
Missouri	2	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	2	1
Maryland	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	.....	1	1
Virginia	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	1	1
North Carolina	.....	1	1	.....	.....	1	2	1	.....	.....	.....	6	5
South Carolina	.....	.....	5	.....	1	.....	1	.....	.....	.....	.....	7	3
Georgia	1	1	2	.....	1	2	.....	.....	.....	.....	.....	7	5
Kentucky	2	.....	1	1	.....	.....	1	.....	.....	.....	.....	5	4
Tennessee	.....	.....	.....	.....	.....	.....	1	.....	.....	.....	.....	1	1
Arkansas	.....	.....	.....	.....	.....	.....	.....	.....	.....	1	.....	1	1
Texas	3	.....	1	.....	1	.....	.....	.....	1	5	1	12	6
Total	11	3	10	1	3	4	7	1	1	8	3	52	
Percentage	21	6	19	2	6	8	13	2	2	15	6		

little or no smut. This was especially true previous to this experiment when the seed from the four inoculated heads was kept separate. Even though this did occur, the total percentage of heads of each variety smutted by each race was fairly constant from year to year.

The distinguishing characteristics of the 11 races given in table 1, the varietal source of each, and the state in which the original collections were made are given in table 2.

The 11 races were collected in 15 states as shown in table 3. Race 1 represented 21 per cent of the collections and was the most widespread of the races. It was collected in Georgia, Illinois, Indiana, Kentucky, Michigan, Missouri, and Texas. Race 3 comprised 19 per cent of the collections studied. Race 3 was collected in Georgia, Kentucky, North Carolina, South Carolina, and Texas. Races 7 and 10 comprised 13 and 15 per cent of the collections, respectively. Races 4, 8, and 9 were represented by 1 collection each, from Kentucky, North Carolina, and Texas. More races were represented in collections from Texas than in those from any other state.

DISCUSSION

From the 52 collections of *Ustilago tritici* on soft winter wheats from New York to Texas, 11 physiologic races have been identified on the basis of pathogenicity tests on 10 varieties of winter wheat. Sufficient data are not available to determine completely the distribution of each race, but from the data at hand, it appears that race 1 is the most widely distributed. It was collected 11 times on various varieties.

From the results as given in table 1, it seems that the smut cultures used in this study were genetically stable. On the other hand, from the nature of the life cycle of the fungus it might be that some of the cultures are in

a heterozygous condition and if so, it might possibly not become evident until several generations had been run through the differential varieties.

Some of the races seem to be limited in distribution. This being the case, care should be exercised in the interchange of seed as a precautionary measure against introducing races into areas where they do not already occur.

On the basis of the results obtained, it should be possible to breed soft wheat varieties for the eastern area that would have desirable agronomic characteristics and also be resistant to the 11 races of *Ustilago tritici*.

SUMMARY

Fifty-two collections of *Ustilago tritici* collected from 15 states of the eastern soft wheat region were tested at the Illinois Agricultural Experiment Station, Urbana, Illinois. Eleven physiologic races were pathogenically distinct and have been assigned numbers from 1 to 11.

Race 1 was the most widely distributed and was collected in 7 of the 15 states. Races 4, 8, and 9 were each represented by 1 collection only. Six different races were collected from Texas; five each from North Carolina and Georgia; four from Kentucky; three from each Illinois and South Carolina; two from Indiana; and one each from the other eight states.

The results presented indicate that it should be possible, by hybridization, to combine resistance to loose smut and desirable agronomic qualities in such a way as to obtain a satisfactory variety of soft winter wheat with a high degree of resistance to all of the 11 races of loose smut.

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CONTROL OF CABBAGE DOWNY MILDEW WITH BENZENE VAPOR[1]

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INTRODUCTION

Commercial cabbage (*Brassica oleracea* var. *capitata* L.) production in Mississippi is centered in Copiah and parts of adjoining counties. The common practice is to sow cabbage seed in coldframes during October and early November, then transplant to the field during late January and February.

Cabbage downy mildew (*Peronospora parasitica* (Fries) Tul.) occurs in this commercial area each year during late fall and winter months. Fungus sporulation is first observed on the cotyledons and later, under favorable conditions, on true leaves. Oospores have been shown to form in the cotyledons (9) and are a possible source of initial infection. Downy mildew or "rust" is the most serious cabbage seedbed disease in Mississippi. In certain seasons this disease becomes epidemic and induces severe losses.

The purpose of this paper is to report experimentation concerned with the development of practical control measures for the control of cabbage downy mildew through the use of benzene vapor. The investigations reported in this paper cover a period of three years, the fall and winter plantbed seasons of 1943–46. All experiments were conducted at the Mississippi Truck Crops Branch Experiment Station, located at Crystal Springs, Mississippi.

LITERATURE REVIEW

The vapors of certain volatile organic materials were used in investigations on downy mildew of tobacco in 1934 by McLean *et al.* (10). Effective results with benzene vapors were reported by Angell *et al.* (1) from Australia in 1935. Subsequent investigation by both Australian and American workers demonstrated the effectiveness of several organic materials on tobacco mildew under both field and laboratory conditions. A review of the major portion of this work is reported by Wolf (12).

Preliminary work with benzene vapors on the control of cabbage downy mildew in Mississippi (7) indicated that both the survival and size of cabbage plants were increased by nightly application of benzene at the rate of 25 cc. per square yard. Pinckard (11) and Foster (6) have described the practical methods of application and results obtained with benzene.

Symptoms of cabbage downy mildew under southern conditions have previously been described (3, 4). The mode of infection of cabbage seedlings was reported (9); also environmental factors affecting the development

[1] Contribution from the Truck Crops Branch Experiment Station, Crystal Springs, Mississippi. Published with the approval of the Director, Mississippi Agricultural Experiment Station, State College, Mississippi. Paper No. 132, New Series.

of cabbage downy mildew (5). A separate report dealing with experimental studies on spray materials for the control of cabbage downy mildew under Mississippi conditions has been prepared.

EXPERIMENTAL PROCEDURE

All experiments were conducted in outside seedbeds or coldframes. Preliminary experiments to determine rates of benzene, number of applications per week, and type of covers were usually conducted in small frame beds, two square yards in area. One 15-gram cotton ball was used to vaporize benzene for each 2 square yards of bed space. Treatments resulting in satisfactory control in the small beds were repeated in larger farm beds.

The seed of several cabbage varieties were used in these experiments. In each experiment a uniform, weighted amount of seed was sown in all seedbed units of equal size. No commercial cabbage varieties tested were found to be resistant to the disease.

Washed, unbleached muslin covers of 48×44 thread count were used in these experiments. This type of cover was the same as or similar to coldframe covers used by many cabbage growers. In some experiments, for comparison, covers with 64×64 thread count were also used. All covers of treated beds, unless otherwise indicated, were saturated with water after the benzene treatments were started.

Small balls of cotton, wrapped in thin muslin and dipped into benzene were suspended by cord from the wooden cover supports. The benzene treatments were made during the late afternoon and beds remained covered during the night. Covers were usually removed after approximately 14 to 16 hours of treatment.

Differential data were obtained by recording total number of plants for one square foot, length of seedlings, weight of plants per one square foot, and weight of 100 plants pulled at random. Weight of plants was based on plant tops, plants being cut at the soil line. A disease rating scale of zero to four was used in recording disease development.

1943–44 EXPERIMENTS

Experiments were set up to determine the rate and number of benzene applications required to control cabbage downy mildew.

Small-Bed Experiments

Eight small beds (2-square-yard units) were sown to Round Dutch cabbage seed on November 11, 1943. Beds were covered with 48×44 muslin cloth. All beds were inoculated with a spore suspension on November 23 and the first benzene application was made approximately 36 hours later. Covers of all treated beds were thoroughly soaked with water in order to better retain the benzene vapors.

Five different treatments were used as shown in table 1. The first benzene treatment was applied on December 28, 1943. Beds receiving treat-

ments No. 3 and No. 4 received a total of 35 benzene applications. Beds receiving treatment No. 2 received 12 benzene applications, while beds receiving treatment No. 1 received a total of 13 benzene applications. Final data taken on December 29 and 30, 1943, including explanation of disease rating, are given in table 1.

TABLE 1.—*Summary of benzene treatments (1st series) of cabbage seedbeds in the fall of 1943*

Treatment No.	Treatment	No. of beds treated	Av. no. of plants per ½ square foot	Av. length of seedlings[a]	Av. disease rating[b]
				Inches	
1	50 cc. per sq. yd. on 3 consecutive nights, first application 36 hours after inoculation	2	102	3.4	1–2
2	Same as treatment No. 1 except that first application was delayed until sporulation appeared	2	79	2.8	3–4
3	12½ cc. per sq. yd. each night, first application 36 hours after inoculation	1	91	4.2	2–3
4	25 cc. per sq. yd. each night, first application 36 hours after inoculation	1	128	4.9	1
5	Control, no benzene treatment	2	79	2.8	3–4

[a] Based on measurements of 20 plants.

[b] Disease rating.

0 = No infection observed.

1 = Infection present. Slight necrosis and usually slight scattered sporulation.

2 = General, abundant sporulation usually present on cotyledons and/or true leaves, with or without death of cotyledons. Usually no stunting. Occasional plants may be killed.

3 = General and abundant sporulation present or previously noted. Cotyledons mostly dropped. Infection and usually sporulation occurring on one or more true leaves. Stunting of plants, definite kill of some plants.

4 = Disease symptoms similar, or more severe, than for rating 3. Death of a large number of plants. (Extremely severe condition approaching optimum for rapid disease development.)

Mildew development was encouraged by watering beds preceding benzene treatments whenever soil and plants appeared dry. The first fungus sporulation was observed on December 3, 1943, ten days following inoculation. The minimum temperature from December 3 to 28, 1943, beneath the bed cover, varied from 26° to 61° F. The maximum temperature, during the same period, varied from 36° to 66° F.

The results of this experiment, summarized in table 1, show that treatment No. 4, or nightly benzene applications at the rate of 25 cc. per sq. yd., gave the best control and indicated that, under the conditions of this experiment, the degree of control was satisfactory. Only one additional treatment,

No. 1, gave fair control. This treatment, 50 cc. of benzene per sq. yd. for three consecutive nights, was promising for control but under more severe conditions might not sufficiently limit disease development. Treatment No. 2 was definitely unsatisfactory, showing that applications of 50 cc. per sq. yd. on three consecutive nights, starting applications following sporulation, did not control the disease. This fact brings out a striking difference in the effectiveness of benzene control of cabbage and tobacco downy mildew, the latter disease having been satisfactorily controlled with benzene following sporulation. Treatment No. 3, or nightly applications of 12½ cc. per sq. yd., with the first application 36 hours following inoculation, failed to give satisfactory control. The benzene usually vaporized during the early part of the night leaving insufficient vapor to inhibit sporulation during the early morning hours. Some plants were observed to have been killed from mildew in beds receiving treatments No. 2 and No. 5.

As shown in table 1, treatment No. 4 gave an average of 128 plants per ¼ square foot, an average seedling length of 4.9 inches, and an average disease rating of 1. Treatment No. 1 gave an average of 102 plants, 3.4 inches in length, and an average disease rating of 1 to 2. Treatment No. 5, or untreated control, gave an average of 79 plants, 2.8 inches in length, and an average disease rating of 3 to 4.

Farm-Bed Experiments

In addition to experiments conducted in small 2-sq.-yd. beds, information pertaining to benzene control of cabbage downy mildew was obtained from experiments conducted in three large farm beds, each approximately 70 square yards. Bed No. 2 and No. 3 were sown on October 10, 1943. Bed No. 2 contained several different cabbage varieties while bed No. 3 was sown to Detroit Resistant. Bed No. 4 was sown on November 11, 1943, and contained several cabbage varieties. Small untreated control beds were sown to Round Dutch cabbage seed on each of the above mentioned dates.

All beds were covered with muslin cloth of 48×44 thread count. Covers of treated beds were thoroughly wet following benzene treatments. Sporulation, from natural infection, developed in each bed in advance of benzene treatments.

In beds No. 2 and No. 4 benzene was applied at the rate of 50 cc. per sq. yd. In bed No. 3 benzene was applied at the rate of 25 cc. per sq. yd.

Benzene applications, for beds No. 2 and No. 3, were applied as mildew sporulation indicated the necessity of maintaining practical control, usually 3 successive treatments per week. In bed No. 4 three successive treatments per week were applied regularly, following sporulation.

The first sporulation, in bed No. 4, was observed on December 9, 1943, and the first benzene treatment was applied on December 10. On January 12, 1944, the 21st and final benzene treatment was applied. During December considerable disease developed on cabbage plants in bed No. 4, especially during nights when benzene treatments were not applied, and there was definite stunting and death of some plants.

Bright sunny weather generally prevailed during November and early December and was conducive of only mild disease development. On December 1, 1943, bed No. 2 received the 10th and final benzene treatment; and bed No. 3 received the 13th and final treatment. At this date cabbage plants had developed the 4th and 5th true leaf. Only slight scattered sporulation was present in either treated or untreated beds and severe mildew infection later in the season was not anticipated. Later in December following the termination of the experiment and during a period of cold, rainy weather, beds No. 2 and No. 3 were necessarily covered for several days. During this period mildew sporulation advanced from the lower true leaves to the upper younger leaves. In some places soft rot (as a secondary infection) followed downy mildew. As a result of the two diseases many cabbage plants were killed. This late attack suggested the desirability of continuing benzene treatments approximately to transplanting time.

Results shown in table 1, as well as observational data on large farm beds No. 2, 3, and 4, strongly suggest that benzene applied on three successive nights and at the rate of either 25 cc. or 50 cc. per sq. yd. will not satisfactorily control cabbage downy mildew. These results also suggest that benzene may be less effective fungicidally in the control of cabbage downy mildew than in the control of tobacco downy mildew. Good control of tobacco downy mildew has been obtained, following sporulation, by treating with benzene on 2 to 3 successive nights per week.

1944–45 EXPERIMENTS

Small-Bed Experiments

During the second season of experimental work on the control of cabbage downy mildew, experiments were outlined to further investigate control measures in relation to rate and number of benzene applications. In addition, thread count of bed covers in relation to effective control was considered.

Series 1. Eight different treatments were used as shown in table 2. The first series of small beds were sown with Copenhagen Market cabbage seed on October 19, 1944. Seedbeds were watered in advance of seeding and again following seeding to insure better germination and a more uniform emergence. All 12 beds were inoculated on October 30, 1944, with a spore suspension of the downy mildew fungus. The first benzene treatments were applied approximately 36 hours following inoculation. Mildew development was encouraged by watering beds ahead of benzene treatments, whenever the soil surface appeared dry. All treated beds, with the exception of No. 5, received a total of 52 treatments. The bed receiving treatment No. 5 was given 38 benzene treatments. The final benzene treatment was applied on December 21, 1944. Final data were taken twelve days later, on January 2, 1945, as shown in table 2.

Even with regular watering of beds the mildew attack was not sufficiently severe to kill a high percentage of plants in the untreated control beds.

Plant stand was not particularly uniform as indicated in table 2, and final data failed to give an accurate picture of earlier mildew development. Disease development taken at two different dates for this first series showed marked differences between better treatments and the untreated control.

The main conclusion that can be drawn from this experiment is that the

TABLE 2.—*Summary of benzene treatments (1st series) of cabbage seedbeds in the fall of 1944*

Treatment No.	Treatment	No. of beds treated	Av. no. of plants per 1 sq. ft. (large plants)	Av. wt. of plants per 1 sq. ft. (above soil line)	Av. disease rating[a] Nov. 22, 1944
				Grams	
1	50 cc. per sq. yd. each night under 64 × 64 cover, dry	1	82	285	1
2	50 cc. per sq. yd. each night under 64 × 64 cover, wet	1	99	241	1
3	50 cc. per sq. yd. each night under 48 × 44 cover, dry	1	90	246	2
4	50 cc. per sq. yd. each night under 48 × 44 cover, wet	1	75	280	1
5	50 cc. per sq. yd. on 5 successive nights, skip 2 nights, then repeat treatments, under 48 × 44 cover, wet	1	66	220	1–1.5
6	25 cc. per sq. yd. each night under 48 × 44 cover, wet	2	75	249	2
7	12½ cc. per sq. yd. each night under 48 × 44 cover, wet	2	80	222	2.5
8	No benzene treatment, cover dry each night, 48 × 44 cover	2	85	156	3
8–A	No benzene treatment, cover dry each night, 64 × 64 cover	1	61	126	3

[a] Disease rating. See table 1, footnote b.

higher rates of benzene, 50 cc. per sq. yd., and under both grades of covers induced better control than the lower rates of benzene.

Series 2. Earlier experiments indicated that nightly applications of benzene at the rate of both 50 cc. and 25 cc. per sq. yd. gave fair control. Consequently both rates were again compared under two types of covers, both wet and dry. In this experiment all benzene treatments were applied on 5 successive nights per week.

Ten small (2-square-yard) beds were sown to the Golden Acre variety on November 16, 1944. All beds were inoculated with a spore suspension

on November 28 and the first benzene applications were made November 29, 1944. Six different treatments were used (Table 3), two beds for each treatment. A total of 47 benzene applications was made for each treatment, two treatments during December having been omitted. The final benzene treatment was applied on January 26, 1945, and final data were taken 19 days later on February 14, 1945.

Data given in table 3 show that downy mildew reduced the stand of cabbage plants in untreated control beds. The average weight of plants (above soil line) per square foot was markedly less for plants from untreated beds; also the weight of 100 plants from untreated beds was strikingly lower than the weight of an equal number from treated frames. The best control resulted from treatment No. 1, or 50 cc. per sq. yd. on 5 successive nights under 64 × 64 dry cover, and No. 3, or 50 cc. per sq. yd. on 5 successive nights under

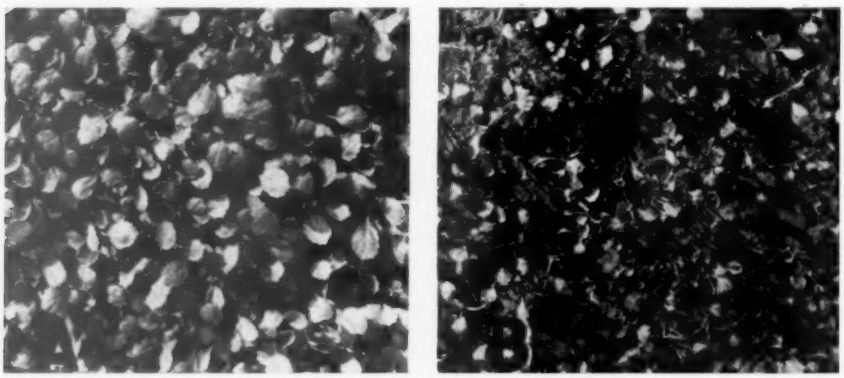

FIG. 1. Plants from Series 2 (2-sq.-yd. beds) 1944–45. A. Treated with benzene at 50 cc. per sq. yd. on 5 successive nights per week under a wet cover of 48 × 44 thread count. B. Untreated control under a wet cover of 48 × 44 thread count. Note sparse stand and stunted plants in untreated control. Approximately same magnification. Photographs taken January 4, 1945, by E. L. Moore.

48 × 44 wet cover. However, differences in stand count and weights between treatments, as shown in table 3, were not markedly high for any one treatment.

Beds receiving treatments No. 1 and No. 3 produced approximately the same average number of plants per square foot, 169 and 171 respectively. Treatments No. 2 (50 cc. per sq. yd. on 5 successive nights under 48 × 44 dry cover) and No. 4 (25 cc. per sq. yd. on 5 successive nights under 48 × 44 wet cover) were less effective in controlling mildew than either treatment No. 1 or No. 3, but gave higher stand counts. Treatment No. 3 is probably the safest treatment of the group and with respect to average weight of plants per square foot was better than other treatments, indicating more vigorous plant growth. The difference in appearance between plants receiving treatments No. 3 and No. 5 (untreated control) is illustrated in figure 1, A and B. When comparison is made of the average weight of 100 plants, data show that with No. 2, one of the poorer treatments, the value is definitely high.

The other benzene treatments, comparing average weight of 100 plants, are relatively close. One possible explanation for these variable results might be the irregular stand within the beds. Although the same weight of seed was sown in each bed, plants were not uniformly and equally spaced. The principal conclusions that should be drawn from this experiment are: (1) All benzene treatments used, under the conditions of this experiment, resulted in both increase of stand and weight over the untreated controls. (2) Treatments No. 1 and No. 3, based on record of disease development, were superior to other benzene treatments tested in this experiment.

TABLE 3.—*Benzene control of cabbage downy mildew (series 2) in 1944–45*

Treatment No.	Treatment	Av. no. of plants per 1 sq. ft. (large plants)	Av. wt. of plants per 1 sq. ft. (above soil line)	Av. wt. of 100 plants pulled at random (above soil line)	Av. disease rating[a] Jan. 13, 1945
			Grams	*Grams*	
1	50 cc. per sq. yd. 5 successive nights 64 × 64 cover, dry	169	292	226	1.5
2	50 cc. per sq. yd. 5 successive nights 48 × 44 cover, dry	195	270	257	2–2.5
3	50 cc. per sq. yd. 5 successive nights 48 × 44 cover, wet	171	342	233	1.5
4	25 cc. per sq. yd. 5 successive nights 48 × 44 cover, wet	173	289	222	2–2.5
5	No benzene treatment, 48 × 44 cover, dry	155	171	135	3–3.5
6	No benzene treatment, 64 × 64 cover, dry	138	197	158	3–3.5

[a] Disease rating. See table 1, footnote b.

Experiments with Large Farm Beds

The object of these experiments was to gain additional information regarding benzene control in cabbage beds similar to those used by commercial truck farmers.

Experiment 1. Four large farm beds were used in these experiments, each bed approximately 66 square yards. On September 25, 1944, the beds were treated with chloropicrin at the rate of approximately 10 lb. per 66 sq. yd. Beds Nos. 4 and 5 were sown on October 17 and beds Nos. 6 and 7 on October 18; all four beds were sown to the variety Copenhagen Market, one lb. seed per bed. Muslin of 48 × 44 thread count was used to cover beds No. 4 and No. 5 while 64 × 64 muslin was used to cover beds No. 6 and No. 7. These four beds were spot-inoculated with a spore suspension on October 30 and again on November 4. Beds No. 4 and No. 7 were used as untreated

controls while beds No. 5 and No. 6 were benzene treated at the rate of 50 cc. per sq. yd. The first benzene treatment was applied October 31, ap-

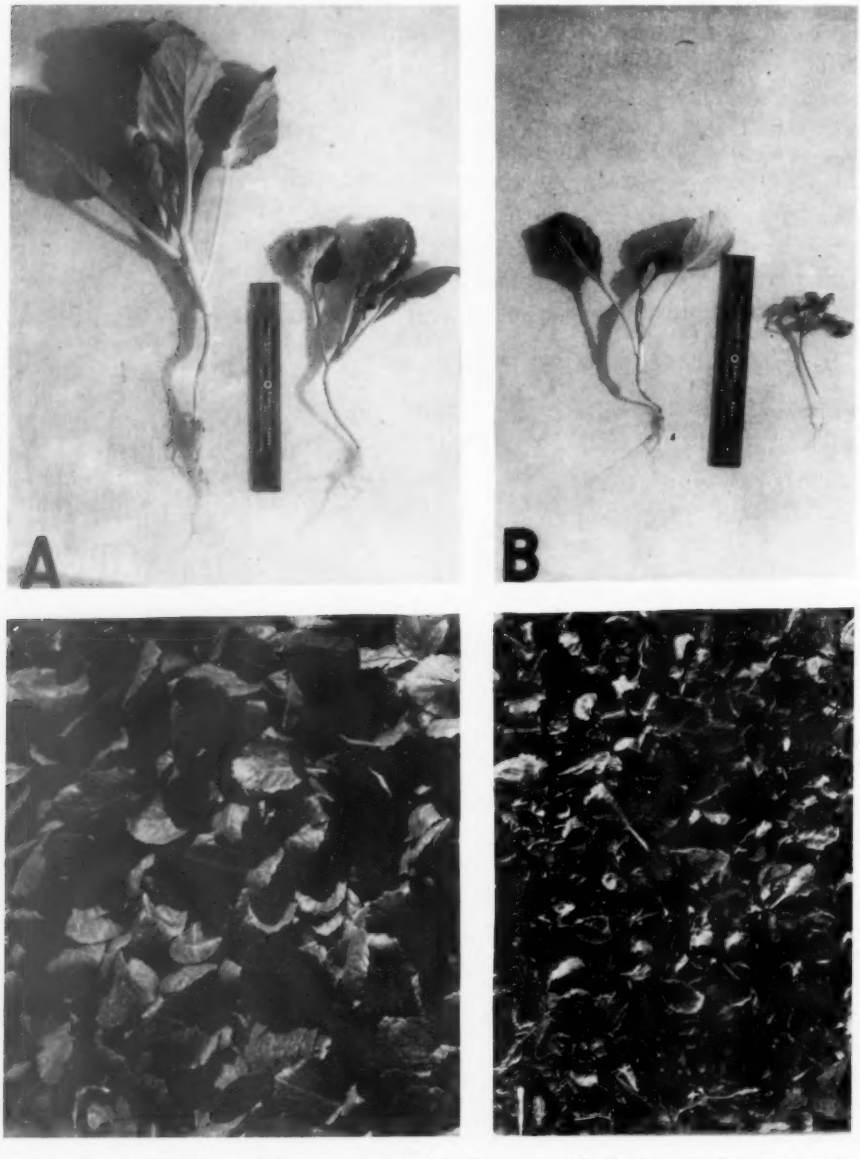

FIG. 2. Comparison of benzene treated and untreated plants. A. Representative large plants from farm bed No. 5 (benzene treated) and farm bed No. 4 (untreated). B. Representative small plants from farm bed No. 5 (benzene treated) and farm bed No. 4 (untreated). C. Section from benzene treated farm bed No. 5. D. Section from untreated farm bed No. 4. Approximately same magnification. A and B taken on January 4, 1945; C and D taken on November 27, 1944 by E. L. Moore.

proximately 36 hours following inoculation. A total of 38 benzene applications were made for beds No. 5 and No. 6. The final benzene treatment was

TABLE 4.—*Relation of cabbage seedbed cover to benzene control of downy mildew in farm seedbeds, 1944–45*

Bed No.	Treatment	Av. no. of plants per 1 sq. ft.	Av. wt. of plants per 1 sq. ft. (above soil line)
			Grams
6	50 cc. per sq. yd., 64 × 64 cover, wet	73	340
7	Untreated control, 64 × 64 cover, dry	66	220
5	50 cc. per sq. yd., 48 × 44 cover, wet	112	392
4	Untreated control, 48 × 44 cover, dry	81	194

applied December 21, 1944. Final data were taken eight to eleven days later. Results of these experiments are shown in tables 4 and 5.

Data shown in table 4 are from beds No. 4, 5, 6, and 7. These data indicate an increase in number of plants per square foot in benzene treated bed No. 5 over the untreated control beds. The weight of plants per sq. ft. was in favor of benzene treatments, 340 and 392 grams compared with 220 and 194 grams. Differences between treated and untreated plants from farm beds No. 4 and No. 5 are shown in figure 2.

Table 5 compares plant stand and weight per square foot in relation to plant size, small, medium, and large, from beds No. 6 and No. 7. The number of plants per square foot was again variable but the average was in favor of the treated bed, 98 compared with 83 from the untreated control. The average weight of plants per square foot was markedly higher for the benzene treated bed No. 6, 227 grams compared with 157 for the untreated control. These results suggest that with downy mildew control larger and stockier plants developed. These data again suggest that plant stand was more or less variable and taken alone was not entirely reliable in indicating

TABLE 5.—*Plant size in relation to benzene control beneath 64 × 64 muslin*

Seedbed No.	Treatment	Plant size	Plants per 1 sq. ft.	Av. plants per 1 sq. ft.	Wt. of plants per 1 sq. ft.	Av. wt. of plants per 1 sq. ft.
			Number	*Number*	*Grams*	*Grams*
6	Benzene, 50 cc. per sq. yd., 64 × 64 cover, wet	Large	98		324	
6	Do	Medium	89		217	
6	Do	Small	106	98	138	227
7	No benzene, 64 × 64 cover, dry	Large	71		230	
7	Do	Medium	106		150	
7	Do	Small	72	83	92	157

the degree of downy mildew control. Differences in plant size from benzene treated bed No. 5 and untreated bed No. 4 are illustrated in figure 3.

During this experiment many cabbage plants receiving benzene treatment (50 cc. per sq. yd.) beneath a 64 × 64 cover appeared abnormal, apparently because of benzene injury. Late in the season, during December, 1944, many plants showed marked chlorosis on one or more leaves. Plants appeared somewhat stunted, with shortened internodes, and with a general flattened appearance. The difference in appearance between cabbage plants in bed No. 5 and bed No. 6 was striking, as shown in figure 3. Similar symptoms, apparently from benzene injury, were observed in small (2-square-

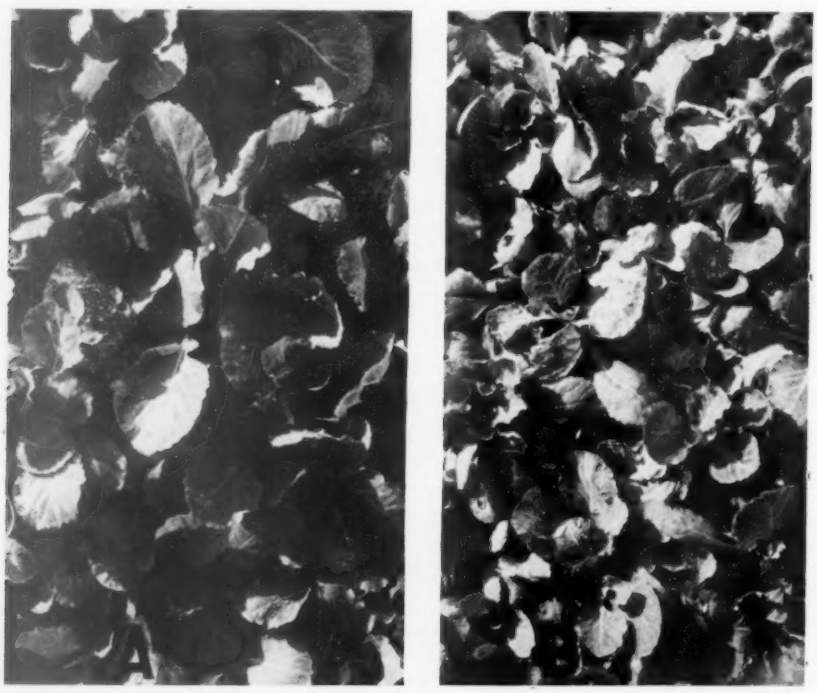

FIG. 3. A. Section from farm bed No. 5 treated with benzene at 50 cc. per sq. yd. on 5 successive nights per week under a wet cover of 48 × 44 thread count. Note vigorous healthy plants. B. Section from farm bed No. 6 treated with benzene at 50 cc. per sq. yd. on 5 successive nights per week under a wet cover of 64 × 64 thread count. Note flattened distorted plants as a result of benzene injury. Approximately same magnification. Photographs taken January 4, 1945, by E. L. Moore.

yard) beds covered with 64 × 64 muslin and receiving 50 cc. of benzene per sq. yd. These small beds were in the 2nd series of 1944–45.

Experiment 2. On November 17, 1944, two large farm beds, approximately 66 square yards, were sown to the Golden Acre variety. Both beds were covered with 48 × 44 muslin. Downy mildew spore inoculation was made on November 28, 1944. Bed No. 8 received benzene treatments, 25 cc. per sq. yd., on 5 successive nights per week while bed No. 9 was used as the untreated control. The first benzene application was made 36 hours after

inoculation. A total of 42 benzene treatments were applied to bed No. 8, three regular treatments being omitted during December. The final benzene application was made on January 30, 1945. Final data were taken 16 days later on February 15, 1945 (Table 6).

Notes pertaining to disease development were taken during the course of the experiment. On December 19, 1944, very little fresh sporulation was observed in either the treated bed No. 8 or the control bed No. 9. On December 30, 1944, following a period of cloudy rainy weather (high humidity) abundant fresh sporulation was observed in the control bed No. 9 with only a trace to slight sporulation in the treated bed No. 8. Sporulation was observed to be more pronounced in low areas of bed No. 8 where drainage was poor and a higher humidity had maintained. Again on January 13 fresh sporulation was observed in control bed No. 9, with moderate to severe

TABLE 6.—*Benzene treatment (25 cc. per square yard) in relation to plant stand and weight*

Bed No.	Benzene treatment	Av. plants per 1 sq. ft. (large plants)	Av. wt. of plants per 1 sq. ft. (above soil line)	Av. wt. of 100 plants pulled at random (above soil line)
			Grams	*Grams*
8	25 cc. per sq. yd. 5 successive nights per week under 48 × 44 cover, wet	116	232	251
9	Untreated control	94	169	219

necrosis on true leaves, slight stunting, and a few plants killed from mildew. In the treated bed No. 8 general moderate necrotic flecking was apparent on nearly all true leaves but with only an occasional plant killed from mildew. In general, plants appeared stronger and in better condition in bed No. 8 than in bed No. 9. However, the benzene treatment of 25 cc. per sq. yd. appeared to be barely holding the downy mildew disease from becoming serious.

In table 6 final data are given based on results from beds No. 8 and No. 9. Results are in favor of the benzene treated bed, indicating that during the late fall and early winter, when conditions are frequently favorable for mildew development, benzene treatments at the lower rate of 25 cc. per sq. yd. may, to some extent, check the downy mildew disease.

1945–46 EXPERIMENTS

During the third seedbed season experimental work was continued in relation to rate of benzene to type of coldframe cover required for adequate mildew control.

Early Round Dutch seed were sown on October 31, 1945, at a uniform rate to each of the small (2-square-yard) beds. Spore inoculation was made

on November 11 and the first benzene treatment was applied on November 12, 1945. The first sporulation was observed on November 18, 1945. The final benzene treatment was applied January 11, 1946. A total of 42 benzene treatments was applied, three regular treatments being omitted during December. Final data were taken on January 29, 1946.

The benzene treatments compared in this experiment, also selected data including explanation of disease rating, are shown in table 7. During previous experiments the first treatment listed (50 cc. per sq. yd. under a 48 × 44 wet cover) has been found consistently to be the most satisfactory treat-

TABLE 7.—*Comparison of different quantities of benzene and different types of cover in the control of downy mildew on cabbage*

Amount of benzene for 1 sq. yd.; condition and type of cover[a]	Plants in 1 sq. ft. of seedbed	Wt. of plant tops in 1 sq. ft. of seedbed	Wt. of 100 plant tops (large plants pulled at random)	Disease rating[b]
	Number	*Grams*	*Grams*	
50 cc., under a 48 × 44 (thread count) wet cover	105	245	546	1–2
50 cc., under a 64 × 64 (thread count) dry cover	80	133	323	2–3
50 cc., under a 48 × 44 (thread count) dry cover	85	158	468	2
35 cc., under a 64 × 64 (thread count) dry cover	96	165	427	2–3
35 cc., under a 48 × 44 (thread count) wet cover	116	262	557	2
25 cc., under a 48 × 44 (thread count) wet cover	84	226	596	2
25 cc., under a 64 × 64 (thread count) dry cover	99	267	726	1.5
No benzene treatment 48 × 44 (thread count) dry cover	85	149	378	2–3

[a] All treatments were applied on 5 successive nights per week in advance of sporulation.

[b] Disease rating. See table 1, footnote b.

ment under severe mildew conditions. The second treatment (50 cc. per sq. yd. under a 64 × 64 dry cover) was found to induce severe benzene injury. The third treatment (50 cc. per sq. yd. under a 48 × 44 dry cover), as in previous experiments, failed to satisfactorily control sporulation during the early seedbed stage. Both the fourth and fifth treatments (35 cc. per sq. yd. under 64 × 64 dry cover, and 35 cc. per sq. yd. under a 48 × 44 wet cover, respectively) were considered unsatisfactory. The fourth treatment, with the 64 × 64 cover, induced benzene injury; roots of many cabbage plants had decay and weight of plants was relatively low. The fifth treatment failed to satisfactorily limit mildew sporulation during the seedling stage. The sixth treatment (25 cc. per sq. yd. under a 48 × 44 wet cover) also failed to control mildew sporulation. The seventh treatment (25 cc. per sq. yd.

under a 64 × 64 dry cover) appeared promising. Cabbage plants grown under this treatment were especially large and vigorous. Although the stand count per square foot was not particularly high, the weights, both per square foot and per 100 plants, were the highest for any treatment. The untreated control was markedly low for stand count and weights. With only one treatment, No. 3, were there fewer plants and lower weight of plant tops per square foot.

This experiment included the first trial using the 25 cc. rate under a 64 × 64 (thread count) dry cover. Sporulation was satisfactorily controlled in this trial and plants were of excellent size and condition. Although promising, additional experiments, including replicated trials, are essential before this treatment can be recommended to cabbage growers.

COST OF BENZENE CONTROL

Cost of control measures must be considered before practical recommendations can be made. Basing cost of benzene on experimental farm bed treatments for 1944–45, benzene at the rate of 50 cc. per sq. yd. would not exceed 50 cents per thousand plants. If the rate of benzene during the latter half of the seedbed season was reduced to 25 cc. per sq. yd. the cost of benzene should not exceed 30 cents per thousand plants. Since plants grown out of the State and trucked into Mississippi frequently sell at $2 to $4 per thousand, the cost of benzene would not be prohibitive.

DISCUSSION

Environmental factors are of primary importance in relation to the development of cabbage downy mildew. In comparing hygro-thermograph records with observations on sporulation and disease development, high humidity has always been of major importance. Sporulation has been observed to occur within a wide temperature range, from approximately 35° to 80° F., providing high humidity approaching a saturated atmosphere occurred during the night. These observations are in close agreement with experimental data presented by other investigators (3, 5).

Experiments conducted during the 1943–44 seedbed season showed that nightly applications of benzene at 25 cc. per sq. yd. resulted in markedly better plants when compared with untreated cabbage beds. Observational data indicated benzene treatments should be continued to within a few weeks of field transplanting.

During the 1944–45 seedbed season 50 cc. of benzene per square yard on 5 successive nights under a 48 × 44 wet cover gave relatively good control. The average disease rating with this treatment was always low and average weight of plants per one square foot was usually high. Under artificially severe mildew conditions this treatment kept downy mildew sporulation under good control.

During the 1945–46 seedbed season benzene treatments at the rate of 50 cc. per square yard on five successive nights per week under a 48 × 44 wet

cover continued to give satisfactory control. Several additional benzene rates were tested under both 48 × 44 and 64 × 64 covers. One treatment, 25 cc. per sq. yd. under a 64 × 64 dry cover, appeared promising. Plants in this treatment were especially large and vigorous, also practically free from downy mildew.

Benzene treatments at the rate of 25 cc. per square yard under a 48 × 44 wet cover frequently failed to satisfactorily control mildew sporulation during the early seedling stage. However, after cabbage plants developed the third true leaf this lower benzene rate did hold mildew sporulation in check.

Benzene applications at the rate of 50 cc. per square yard, under a wet cover of 64 × 64 muslin, induced injury on cabbage plants which became apparent during the latter part of the plant bed season. These results suggest that benzene may be more highly toxic to the cabbage plant than to the tobacco plant. Wolf *et al.* (12) stated that "even when amounts of benzol lethal to the pathogen are applied it becomes very difficult to injure tobacco seedlings under field conditions since the limits of toxicity of benzol to the parasite and to the host are widely separated." Clayton *et al.* (2) stated that the "immediate plant injury from benzol treatment can only result from extreme neglect, as the margin of safety is very great."

Evidence presented by Wolf *et al.* (12) and Clayton *et al.* (2) show that benzene satisfactorily controlled tobacco downy mildew following the appearance of active sporulation. Clayton *et al.* (2) indicated that with covers of 40 and 46 thread count a fair degree of control was obtained and control was markedly improved by increasing the thread count. Benzene experiments with cabbage mildew started following abundant general sporulation and under relatively favorable mildew conditions have failed to satisfactorily control mildew infection.

Three years of experimentation in the control of cabbage downy mildew justify the following conclusions: Benzene at the rate of 50 cc. per square yard should be applied on five successive nights per week under a 48 × 44 wet muslin with applications starting in advance of sporulation. Following development of the 3rd or 4th true leaf benzene applications could safely be reduced to 25 cc. per sq. yd. during the remainder of the treatments. To insure adequate control, benzene treatments should be continued until two to three weeks preceding transplanting to the field. Lower air temperatures during the latter part of the seedbed season make it feasible to reduce the benzene rate. Both benzene vaporization and mildew sporulation are markedly reduced when nightly temperatures, under the covers, reach a minimum of 35° F.

SUMMARY

1. Observational data indicate that humidity is of primary importance in sporulation of the downy mildew fungus.

2. Results from repeated experiments indicate that benzene is effective at the rate of 50 cc. per square yard when applied on five successive nights

per week under a wet muslin cover of 48 × 44 thread count, with applications starting in advance of sporulation and continuing to within a few weeks of field transplanting.

3. Following development of the 3rd or 4th true leaf benzene applications could be reduced to 25 cc. per square yard during the remainder of the treatments.

4. Benzene at 25 cc. per square yard on five successive nights per week under a 64 × 64 dry cover appeared promising.

5. Data were primarily recorded by taking number and weight of plants per square foot, weight of 100 large plants pulled at random, and disease rating based on a scale from zero to four.

6. Cost of benzene treatments was estimated at approximately 30 cents per thousand plants.

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SEED TRANSMISSION OF RHIZOCTONIA SOLANI IN RELATION TO CONTROL OF SEEDLING DAMPING-OFF

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The possibility that *Rhizoctonia solani* Kühn, a fungus that commonly causes damping-off of seedlings, may be seed borne is disregarded or denied in most plant pathology textbooks and general bulletins (*e.g.*, 3, 5, 16, 22, 23, 26), although there are several reports of such transmission.

Apparently the first record of seed infection by *Rhizoctonia solani* was that of Hedgecock in 1904 (14) on beans. Mycelia and sclerotia were present in the seed coats, having penetrated through pods in contact with the soil. Fulton (11) later showed that such fungi caused damping-off of seedling beans. Its occurrence in bean seed has subsequently been verified (10). Garden peas were shown by Crosier to be infected in 1931–32 and later (7, 8, 10); hairy vetch (9), peanut (10, 12), and subterranean clover seed (6) also have been found to carry *Rhizoctonia*. These records are all on field crops, and it is to be expected that *Rhizoctonia* transmission in such seeds is difficult to detect and regarded as unimportant; it has been observed largely in seed-testing procedures.

This viewpoint has been carried over into the damping-off problem, delaying progress in disease prevention. *Rhizoctonia* is held to be a soil organism, and control procedures are aimed at its elimination or suppression in the soil. In commercial practice, spot infections of damping-off have been attributed to faulty soil treatment or to recontamination, whereas it is now clear that some of them result from infected seed. For plants normally started in flats or beds of treated soil this fact has marked significance.

It is the purpose of this paper to report additional evidence of seed transmission of *Rhizoctonia*, its significance, and methods of prevention, in extension of a previous abstract (2).

SEED TRANSMISSION IN VARIOUS CROPS

Pepper (*Capsicum frutescens* L.).—During the period, 1936–45, there were grown annually in southern California more than 5700 acres of chili and bell peppers and pimientos. The crop is largely started with transplants from outdoor seedbeds and greenhouse flats. The production of these seedlings, mostly during the cool months of December to March, is recognized by nurserymen as difficult and precarious, and heavy losses have been accepted as inevitable. Farmers have contracted with as many as 6 nurserymen to insure the required number of seedlings. These losses are primarily due to damping-off caused by *Rhizoctonia solani*. Similar difficulties are encountered elsewhere (4, 15, 31).

Soil pasteurization reduced losses from damping-off in commercial nurseries, but spot infections continued to appear in many flats of seedlings.

Tests with the same seed lots were conducted in the University greenhouses. The seed was sown in soil carefully pasteurized in the flats, and was held in a greenhouse protected from contamination. Again *Rhizoctonia* damping-off developed. Some of the seed was placed on sterile black peat in Petri dishes in the laboratory and after a week typical *Rhizoctonia* mycelium was observed growing out from the seeds and from bits of plant debris which accompanied them.

Further studies showed that most, but not all, seed lots carried this fungus, and not uncommonly had 0.3 per cent infected seed. It was later found that seed from individual fruits may have a much higher percentage of infection. For example, seed from a rotting fruit was treated with sodium hypochlorite (0.5 per cent available chlorine) and the seed coats carefully removed. The contents were planted on agar and all developed *Rhizoctonia*, which indicated internal infection of every seed.

Since much of the seed used locally was grown in Orange and San Diego counties, California, the field conditions which gave rise to such infection were investigated. It was found that some of the seed fields had been planted several successive years to peppers. The plants were very vigorous and were bearing so many large fruits that branches frequently were split or bent down by the weight, the fruit continuing to develop on the ground. Heavy applications of mineral fertilizer were customarily used. Large quantities of water were applied by ditch irrigation, and, because of the dense interlocking top growth, the soil surface was almost continuously wet. Under these conditions it was not surprising to find abundant *Rhizoctonia* soil rot of the fruit, as described for pepper (31), tomato (24, 25), and beans (11, 14, 18). Since peppers grown for seed remain on the plant until they start to wither, the losses from rotted fruit are frequently severe. From the standpoint of seed transmission, however, it is partially decayed fruit which is important.

The ripe fruit is picked and run through a chopper to reduce it to small bits. The pulp is placed in large barrels for several days to decompose, and then run through a long sluice box in which the seed settles to the bottom. The seed is dried on screens and later cleaned by machinery to remove most of the extraneous matter. This whole procedure gives ample opportunity for the *Rhizoctonia* mycelium to spread and invade the seeds. It is not surprising that sclerotia of *Sclerotinia sclerotiorum* (Lib.) Schroet. were sometimes found mixed with the seed.

Histological studies were made to determine the location of infection. Bits of seed were embedded in paraffin, and sections 8–12 μ thick were cut. These were stained with safranin in 50 per cent alcohol and counterstained with fast green in 95 per cent alcohol. It was found that infection of the seed may be present at any of the following points:

1. On the surface and in cells of the outer layer of the testa. The mycelium grows over the surface of the seed and may form small sclerotia there (Fig. 1, B, G). Occasionally it penetrates into the lumen of, but probably not through, the lignified cells of the outer layer of the testa.

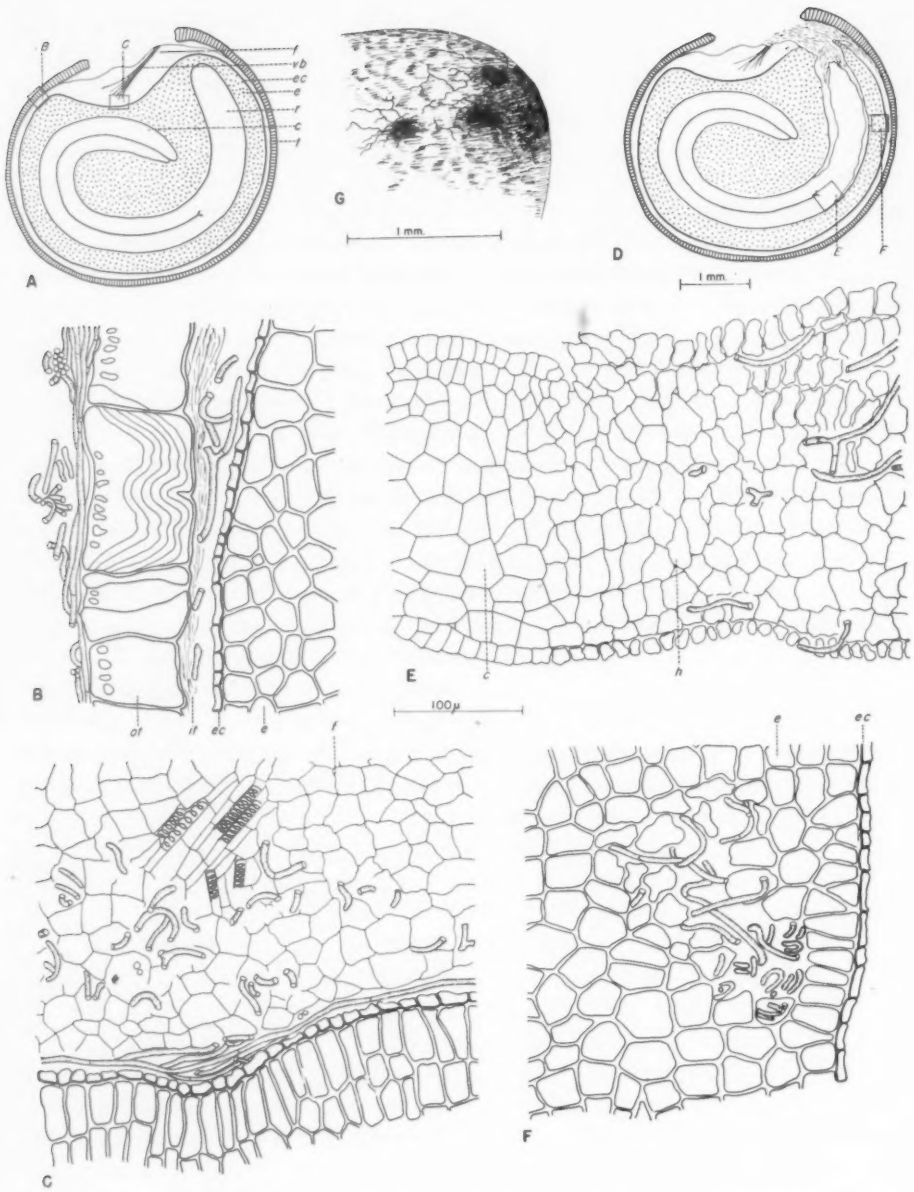

FIG. 1. Camera lucida drawings of bell pepper seed infected by *Rhizoctonia solani*. A–C. Seed with infection of funiculus and testa. B. Mycelium on exterior and in inner layer of testa. C. Mycelium in remnant of funiculus. D–F. Seed with infection of embryo and endosperm. E. Mycelium in hypocotyl. F. Mycelium in endosperm. G. Surface of seed coat, showing mycelium and small sclerotia. c = cotyledon; e = endosperm; ec = endosperm cuticle; f = remnant of funiculus; h = hypocotyl; it = inner layer of testa; ot = outer layer of testa; r = radicle; t = testa; vb = vascular bundle of funiculus. Drawn by K. C. Baker.

2. In crushed cells of the inner layer of the testa (Fig. 1, B). Mycelium enters this area through the funiculus and the opening in the seed coat (Fig. 2, A), and may spread there to surround completely the endosperm cuticle. Examination of dried pepper seed under 72 × magnification will reveal the avenue of such penetration.

3. In the remnant of the funiculus (Fig. 1, C). The pathogen grows into the placenta from the pericarp in contact with the soil, and from the placenta extends into the funiculus. If the mycelium is stopped at this point by the endosperm cuticle (Fig. 1, C), it is still carried with the seed,

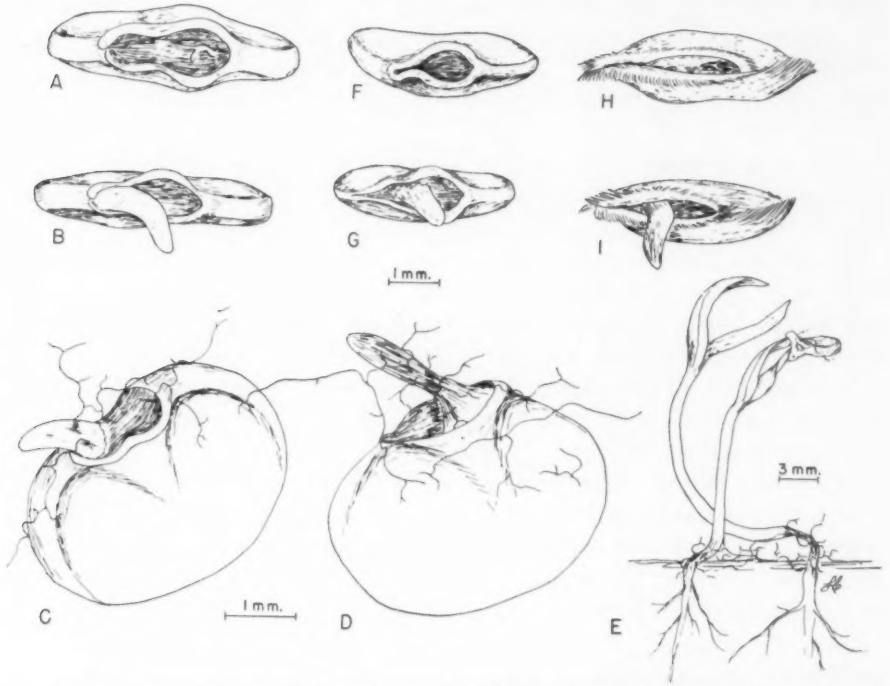

FIG. 2. External seed structure showing opening in testa and dried funiculus through which radicle emerges in germination. A–D. Pepper seeds in various stages of germination and *Rhizoctonia* pre-emergence damping-off. E. Post-emergence damping-off of pepper seedlings. F–G. Eggp ant seed germination. H–I. Tomato seed germination. Drawn by L. H. Clark.

and the emerging radicle must push through this infected tissue at germination (Fig. 2, B, C, D). Infection may occur later from seed coats fallen to the ground (Fig. 2, E).

4. In the embryo and/or endosperm (Fig. 1, E, F). Penetration of these structures may occur (a) through the funiculus before it is sealed off by the delayed cutinization of the outer layer of the endosperm, or (b) through a rupture of the endosperm cuticle possibly by growth pressures. In the first type the seed probably would decay, and might be removed during the commercial cleaning process. In type (b) the crack appears in the cuticle of the thin endosperm tissue over the radicle apex, and the radicle and endo-

sperm are invaded. In this circumstance the seed may be dried before there is enough decay to prevent later germination, or rot may proceed so far that no germination occurs; in either case the fungus is carried to the seedbed where it will spread to adjacent seedlings.

In addition, pentration of the developing ovule or young seed prior to lignification of the testa may occur at any point on its surface and result in complete decay.

Although the testa does not completely cover the seed, even at maturity (Fig. 2, A), a cutinized layer forms around the endosperm during development of the seed.[1] This probably explains why infections are so common in the remnants of the funiculus and inner layers of the seed coat, but less so in endosperm and embryo. It was found, by inoculating fruit at different stages of maturity, that seed transmission is possible even after the seeds have matured.

The fungus appears to digest cells of the embryo or endosperm, at the most, only slightly in advance of the mycelium (Fig. 1, E, F), as found by others (28, 29). The mycelium is both inter- and intra-cellular.

Because of the resistance of *Rhizoctonia* mycelium to drying (13, 26, 29), survival in seeds is to be expected. In the present studies, seed was removed from various pepper fruits infected naturally or artificially, washed, dried, and held in seed packets in the laboratory. Seeds harvested in November, 1943, still developed colonies of *R. solani* when planted on sterile black peat 43 months later in May, 1947, although the mycelium in many of the seeds failed to grow.

Tomato (*Lycopersicon esculentum* Mill.).—Tomato seedlings sustain relatively less damping-off than peppers in southern California, and no evidence of *Rhizoctonia solani* in commercial seed has been obtained. Soil rot of the fruit (24, 25) occurs commonly in seed fields, but the method of seed cleaning is less favorable to spread and growth of the fungus than is the case for pepper. It is probable also that the lower susceptibility of tomato than pepper seedlings plays some part in this situation.

Seed cleaned from inoculated fruit in November, 1944, still developed a few colonies of *Rhizoctonia* on sterile black peat 31 months later in May, 1947. Histological studies revealed mycelium in the remnants of the funiculus and the inner layer of the testa, much as in pepper. There is a much smaller opening in the seed coat of tomato (Fig. 2, H, I) than in pepper, and this may contribute to the lesser importance of *Rhizoctonia* transmission in tomato.

Eggplant (*Solanum melongena* L.).—Eggplant seedlings are started much as are peppers, and frequently sustain heavy losses from *Rhizoctonia* damping-off. Examination of fruit in the field showed some with *Rhizoctonia* soil rot (25) similar to that of pepper, furnishing a possible explanation of infected seed. Fruit inoculated with pepper isolates of *Rhizoctonia*

[1] Baker, K. C. Seed anatomy and ontogeny of Capsicum frutescens L. Unpublished manuscript.

decayed and gave infected seed. Commercial seed of eggplants indexed as for pepper gave clear indications of carrying *Rhizoctonia*.

The opening in the seed coat is similar to that of pepper (Fig. 2, F, G) and it is probable that mycelium also is carried in remnants of the funiculus of these seeds.

Zinnia (*Zinnia elegans* Jacq.).—Zinnias generally are sowed in place, but also are started in flats for nursery sale. Seedlings do not usually sustain losses from *Rhizoctonia* damping-off. It was, therefore, of interest that *Rhizoctonia* should be found in routine testing of zinnia seed.

Commercial zinnia seed lots grown in 3 areas of California were planted in flats of sterile soil in the greenhouse to index for the presence of *Alternaria zinniae* Pape. A number of the lots consistently sustained severe losses from *Rhizoctonia* damping-off in repeated plantings. This was surprising because zinnia flower and seed heads do not normally come into contact with the soil during development.

There are two possible explanations of this phenomenon: (1) In some fields or varieties, plants might be broken or matted down so that flower heads would be in contact with infested soil. It would be unlikely that many such heads would be gathered when hand picking is practiced, but they would not be excluded when the whole plants are put through a threshing machine. (2) Hand-picked flower heads are piled to dry on canvas spread on the ground. Whole plants are sometimes piled on the ground prior to threshing. The bottom layers of either type of pile remain moist and this, plus capillary soil moisture, supplies conditions favorable for the growth of *Rhizoctonia* and infection of the seeds.

SIGNIFICANCE OF SEED TRANSMISSION OF RHIZOCTONIA

The fact that *Rhizoctonia* is seed borne on more kinds of seeds and with greater frequency than is commonly supposed is significant in several ways besides dissemination of the fungus from one area to another (14). Because of the general distribution of *R. solani* in soil, this dissemination has been considered inconsequential, particularly on field crops (8).

Because of the existence of a degree of physiological specialization in *Rhizoctonia solani*, even as it causes damping-off (literature reviewed in 17, 28, 29), dissemination of the fungus with seed assumes more importance. Reciprocal inoculation tests with various isolates from rotting tomato and pepper fruits from commercial fields showed that some infected both tomato and pepper fruits readily, whereas others attacked only one or the other. The concomitant seed carriage provides a means for the continued association of a virulent fruit-rotting *Rhizoctonia* with the appropriate host.

Crops usually started in pasteurized or treated soil probably are most affected by seed transmission of *Rhizoctonia*. It is generally recognized that a pathogen introduced into soil free from competing organisms is able to spread faster and cause more damage than it would in the presence of a normal soil flora (19, 20), although there are exceptions (27). The gross

effect is to make some crops of this type exceedingly difficult to grow unless both soil and seeds are treated.

Since *Rhizoctonia* does not form spores under seedbed conditions, airborne contamination is rare. Bits of mycelium in soil or plant parts may be spattered about in watering, or carried to some extent on tools or flats. Recontamination in these ways can, however, be avoided by careful cultural practices.

CONTROL OR PREVENTION OF SEED TRANSMISSION

Seed treatment.—Because the fungus is carried internally it seemed improbable that chemical treatment would be effective. Since preliminary tests with New Improved Ceresan, Semesan, mercuric chloride, and red copper oxide on infected pepper seed were not promising, hot-water treatment was tried. This proved so effective that no further work was done with chemical treatments.

A hot-water treatment at 51.7° C. (125° F.) for 30 minutes was found to eliminate *Rhizoctonia* seed transmission in chili and bell peppers with slight reduction of germination (greatest reduction with fresh seed was 13.4 per cent). A temperature of 53.9° C. (129° F.) was found to reduce germination so much that it is not recommended. Newton (21) found that cultures of *R. solani* on agar were killed at 50° C. only if exposed for one hour, and that sclerotia on potato tubers were killed by 60° C., but not by 55° C., in one hour. The discrepancy of these data with our results remains unclarified.

Large-scale treatments were begun in 1943, and during 4 seasons many lots, aggregating perhaps a ton of seed, have been treated by us and by nurserymen. In no instance has there been reported any injurious effect and it is concluded, therefore, that the treatment is entirely safe.

In one representative test using 4,000 treated and 4,000 untreated California Wonder Bell pepper seeds, there were 54.6 per cent fewer seedlings suitable for transplanting in the series grown 8 weeks in raw soil free of *Rhizoctonia*, as a result of seed transmission. In the series with untreated seed planted in pasteurized soil there was a 69.5 per cent reduction from those sowed with treated seed. In a commercial nursery 2 to 2½ times as many plants were obtained per seed flat from treated as from untreated seed.

Large lots of seed may be treated in boxes with screen sides. These boxes, about half filled, are plunged into a large tank containing 200–250 gallons of water held by a steam pipe to 51.7° C.; a water pump circulates the water and maintains uniform temperature. No presoak or preheating dip is needed. During the 30-minute exposure the boxes are turned over several times to facilitate uniform heating. At the end of the treatment the boxes are removed, drained, and cooled quickly by flooding with tap water from a hose. The seed is then drained and spread out in thin layers on screens to dry. This may be done outdoors in warm weather, in a heated room with fans, or in a dehydrator, the only requirement being that the seed be dried in 12–20 hours without overheating.

Small lots of seed may be handled in half-filled cheesecloth bags. These are treated as above in a sink or tub of about 30 gallons capacity, temperature being most easily controlled by allowing hot water (60° C., from a water heater) to trickle in so as to hold the temperature at 51.7° C. The bags may be cooled quickly by submerging in cold water or by holding them under the tap.

Only one other recommendation of hot-water treatment of pepper seed has been found (30), using 51.7° C. for 25 minutes, followed by Cuprocide 2½ oz. per 15 lbs., for bacterial spot (*Xanthomonas vesicatoria* (Doidge) Dowson). Hot-water seed treatment was tried (15) for control of pepper diseases in Georgia; its injuriousness probably was due to the 6-hour presoak and high temperature (60° C. for 10–30 minutes) employed. Because of the safety and effectiveness of proper hot-water treatment, it might well be tried against other seed-borne diseases of pepper.

Zinnia seed has been successfully treated the same as peppers, but germination loss may be excessive if the seed is more than a year old. For example, in a test involving 12,000 untreated and 12,000 treated seeds, one-year-old untreated seed of 7 varieties had an average of 65.6 per cent emergence in pasteurized soil, and treated seed 55.4 per cent (a decrease of 15.6 per cent), 2-year-old seed of 4 varieties averaged respectively for untreated and treated lots, 66.0 and 40.3 per cent (a decrease of 38.9 per cent), and 3-year-old seed of a single variety 37.8 and 8.0 per cent (a decrease of 78.8 per cent). Eggplant seed also has been safely treated in the same way.

Cultural practices.—Practices aimed at keeping fruit off of the ground to prevent *Rhizoctonia* "soil rot" will reduce fruit decay. Staking of tomatoes has been suggested (24), and wires stretched on each side of rows of bell peppers grown for seed should reduce fruit decay and seed transmission of *Rhizoctonia*. Sanitation and crop rotation (11) should be of benefit. Careful hand sorting of such large seeds as peas and beans (11) would be desirable. Fungicidal dusts or sprays applied under the plants as for bottom rot of lettuce (29) might be effective, but have not been tried.

In the case of zinnia seed infected from the soil through the canvas on which it is piled prior to threshing, a fungicidal treatment of the canvas might prevent its penetration by *Rhizoctonia*. Several seed growers are using for this purpose canvas treated with mercury or copper compounds for mildew prevention, and this may also prove a practical control of *Rhizoctonia* infection.

IMPROVED GROWING METHODS MADE POSSIBLE BY CONTROL OF DAMPING-OFF

It is possible to reduce damping-off of seedlings in commercial nurseries to insignificance by pasteurization of the soil, hot-water treatment of seed, and reasonably careful handling. This fact has made possible a reexamination of nursery procedures, with improvements in mechanization and labor-saving methods. This is illustrated by improvements adopted since 1944 by one nursery[2] in the raising of pepper seedlings for farm trade.

[2] The excellent cooperation of American Plant Growers, Lomita, California, in this portion of the work is acknowledged with pleasure.

The usual procedure in southern California for raising peppers in flats is to sow "seed flats" thickly, place them on greenhouse benches, hold them fairly dry to reduce damping-off, and transplant to other flats (100–110 plants per 18 × 18 inch flat) when the seedlings are about 1 to 2 inches high. Because some of the plants may have incipient infections when transplanted, and the soil may carry the organism, these flats also are held relatively dry to minimize losses. When the plants are about 10–12 inches high the flats are placed outdoors to "harden." Because seedlings are produced in the winter months, temperatures are likely to be low, and therefore favorable to *Rhizoctonia* infection of such a "warm" crop as pepper. The usual period from sowing to "hardening" is 80 days or more.

Freedom from damping-off has made possible the sowing of seed in place by means of a special machine, since the number of plants is not reduced from disease. This saves the labor of transplanting, the seedlings do not sustain the usual 10–14-day setback from root injury, and the hazard of spreading virus by handling during transplanting is eliminated.

Since the pathogens are destroyed, it is possible to maintain the flats at a high moisture level during germination. Immediately after the seed is planted in the flat and covered with sterile sand, it is rather heavily watered, all of these processes being handled by machines at the rate of 150 flats per hour. These flats are then stacked directly on top of each other and moved into a closed room held at fairly constant temperature and high humidity. Under these moist conditions the seeds germinate uniformly and shed the seed coats without binding. By periodical examination, the flats can be removed from the germination room and placed on greenhouse benches just after emergence, but before the seedlings elongate. By this means greater uniformity and percentage of germination are obtained while avoiding the laborious watering required by seed flats. The greenhouse benches are freed during the 7–14-day germination period, and are used only for plants which require light. This same method of germinating seed flats under conditions free of pathogens is now being successfully used by several nurserymen and seedsmen in California and the mid-West. It has been found useful when a single variety is sowed in numerous flats, but is not desirable where several varieties are involved, because of differences in germination rate. It will be recognized that this method is an adaptation of the practice of covering a flat or pot with a glass plate during germination.

When the seedlings are placed in the greenhouse the humidity may be kept high, and the temperature up to 80° F., with the soil at a high moisture and fertility level. While not yet tried, constant-level watering or subirrigation could be used with still greater reduction of labor. About 50 days are required from seeding to "hardening" with this method of handling.

The flats are moved outdoors for 2 to 3 weeks to harden the plants, during which time most of the leaves abscise and the stems become tough and wiry. The plants are pulled from the flats by hand and, after the dirt is shaken from the roots, placed in celery crates for delivery to the field. Experience

indicates that these plants start rapidly in the field, but with the above method plants of almost any degree of hardness can be produced. It is now generally conceded by farmers who have used the plants that root systems of non-transplanted stock are at least as good as those of seedlings pricked out of seed flats.

The total effect is to produce healthier plants more dependably and quickly and with less labor and expense, in a given greenhouse area. Because the plants are free of virus diseases and such organisms as root-knot nematode and *Rhizoctonia, Pythium, Phytophthora,* or *Sclerotinia,* which cause root, stem, and fruit decay, and of *Verticillium* which causes wilt, the hazard of introducing them to uninfested fields is eliminated. Because of the savings effected by these improved methods, it is probable that plants can now dependably be grown in greenhouses as cheaply as they can with uncertainty in outdoor seedbeds.

It cannot be too strongly emphasized that the success of this method depends on using soil and seed free of pathogens, and on rigorous sanitation, and that without these conditions losses actually may be accentuated.

DISCUSSION

The soil-inhabiting nature of *Rhizoctonia solani* has been so emphasized in phytopathological literature that the traditional control procedures against seedling damping-off aim at elimination or suppression of the fungus in the soil. However, it is now established that the fungus is disseminated in the seed of bean, pea, vetch, peanut, clover, pepper, eggplant, tomato, and zinnia. Such transmission may be the means of reinfesting treated soil, and has resulted in heavy losses in nursery production of pepper plants.

Seed infections of these various crops have in common the fact that they originate from contact of the fruit or flower head with the soil. Pea and bean, and probably vetch and clover, seed are infected on the side through the pod wall. This is analogous to the situation reported for *Macrophomina phaseoli* (Maubl.) Ashby on lima bean (1).

In the case of pepper, eggplant, and tomato, the "soil rot" may spread through the fruit and infect the seed from the placenta. The fungus may penetrate the developing ovule, or young seed with unlignified seed coat, at any point on its surface and produce decay. After the seed coat has lignified, the area of penetration apparently is restricted to the opening through which the funiculus passes. As the endosperm cuticle forms, the area of infection is further restricted. The point of union with the funiculus is the last to be cutinized and, until it has been sealed off, constitutes a bridge to penetration of the endosperm. Thus in the development of the seed there are decreasing areas of possible penetration of the endosperm and embryo by pathogens. However, vulnerable spots remain. The mycelium may grow directly from the funiculus into the inner layer of the testa surrounding the endosperm cuticle; it may remain dormant in this layer and in the funiculus until seed germination, when it infects the emerging

radicle. Growth cracks may develop in the endosperm cuticle, particularly in the region of the tip of the radicle, allowing mycelium to enter the endosperm or embryo. The pathogen may grow over the surface of the seed at any time, forming resting mycelia and sclerotia there, and even penetrating into, but not through, the external cells. *Rhizoctonia solani* has been reported (28, 29) to penetrate the host: (a) between or through epidermal cells (potato, cotton, tomato fruit, lettuce, pea); (b) through cuticle (cotton, kale, lettuce); (c) through lenticels (tomato fruit); (d) through stomata (grass, lettuce); (e) through wounds. Only (a) and (e) seem to be involved in pepper.

In the case of zinnia, there is direct invasion of the seed by fungus growth into the flower heads piled on canvas on the ground. Bits of infected stem, fruit, or other tissues which may accompany seed have the same practical result as infected seed, and are not uncommon in commercial packets.

In all of the examples studied, it is an academic point whether infected seed is killed before planting, or is infected and killed after germination. In either case the mycelium quickly radiates to other seeds and causes pre- and post-emergence damping-off. Equally academic is the question as to whether or not the types of infection described for pepper are all internal. However, it would appear that, to maintain a significant distinction between internal and external seed transmission, the latter must be restricted to cases where detached spores, bits of mycelium, etc., are mechanically lodged on the surface of the testa and grow no further until the seed is planted.

SUMMARY

Seed transmission of *Rhizoctonia solani* has been demonstrated as common in living and dead seeds of bell and chili pepper. The fungus occurs as mycelia or sclerotia on the surface of the seed, or as mycelia in the attached remnants of the funiculus, the inner layers of the seed coat, the endosperm, or the embryo itself, particularly at the tip of the radicle. Infection was found to originate in fruits rotted with *Rhizoctonia* from contact with soil in the field.

Similar seed transmission has been found with eggplant, tomato, and zinnia, and was previously reported for bean, pea, hairy vetch, peanut, and subterranean clover.[3]

Such transmission is significant because: (a) it introduces strains of the fungus to new areas or fields; (b) it contaminates disinfested soil, causing severe seedling loss; (c) it assures the continued association of a virulent strain of the fungus with the appropriate host.

Hot-water treatment of pepper, eggplant, or zinnia seed at 51.7° C. (125° F.) for 30 minutes killed the fungus in and on the seed without significantly reducing germination.

[3] Since this paper was written, reports of two additional examples of seed transmission of *R. solani*, on spinach and Japanese hop, have been found:
Neergaard, P. Aarsberetning fra J. E. Ohlsens Enkes Plantepatologiske Laboratorium (København) **5**: 5. 1940; **6**: 4. 1941; **7**: 6, 14. 1942; **8**: 6. 1943; **10**: 6. 1945.

By treatment of both soil and seed to kill the fungus, coupled with practical sanitation, new techniques for growing seedlings have been developed which give more dependable, faster production of better plants in a smaller greenhouse area than do the usual methods, with less labor and expense, and with reduced chance for carrying virus, fungus, or nematode diseases to the field.

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PHYTOPATHOLOGICAL NOTES

Ceratostomella ulmi on Elm Bark Treated with 2,4-dichlorophenoxyacetic Acid.—Variations in length and diameter of coremial stalks of *Ceratostomella ulmi* (Schwarz) Buisman on elm and in pure cultures have been reported by Clinton,[1] Schwarz,[2] and Wollenweber.[3] The writers have observed such variations in coremia growing in bark beetle galleries in elm and in cultures from bark beetles. The common range in length is 0.25 to 1.5 mm. Wollenweber[3] refers to coremia up to 3 mm. in length and illustrates unusual coremial forms which suggest some similarity to the

FIG. 1. Coremia of *Ceratostomella ulmi* on elm bark treated with 2,4-dichlorophenoxyacetic acid, photographed after 65 days. Large group flattened by cover dish, and few coremia of normal size. Photograph slightly retouched. 4×.

types reported herein. In 1933 the senior writer observed extremely long coremiumlike and apparently sterile synnemata on potato-dextrose-agar slants. Again in 1935 on potato-sucrose-agar, a culture of *C. ulmi*, growing with secondary fungi, showed very abundant overgrowths of large fertile coremia slightly over 3 mm. in length. In one instance in 1946 an atypical culture on nutrient agar medium produced fasciated groups of coremia up to 3 mm. in length. In 1947 when isolates from different geographic locations in the United States were grown together on sterilized elm twigs, coremia from 3 to 4 mm. in length were produced.

[1] Clinton, G. P., and Florence A. McCormick. Dutch elm disease, *Graphium ulmi*. Conn. Agr. Expt. Sta. Bul. 389: 701–750. 1936.

[2] Schwarz, M. B. Die Zweigdurre und die Gefasskrankheit der Ulme. Kapitel II *in* Das Zweigsterben der Ulmen, Trauerweiden und Pfirsichbaume, eine vergleichend-pathologische Studie. 32 pp. Utrecht. 1922. (Transl. by L. D. Kelsey *in* Bartlett Res. Labs. Bul. 1: 5–25. 1928.)

[3] Wollenweber, H. W. Das Ulmensterben und sein Erreger, *Graphium ulmi*, Schwarz. Nachrichtenbl. f. den Deut. Pflanzenschutzdienst, No. 10. 1927. (Transl. by L. D. Kelsey *in* Bartlett Res. Labs. Bul. 1: 26–31. 1928.)

In September, 1946, while using chemicals in an attempt to retard growth of secondary organisms and to hasten coremia formation of *Ceratostomella*

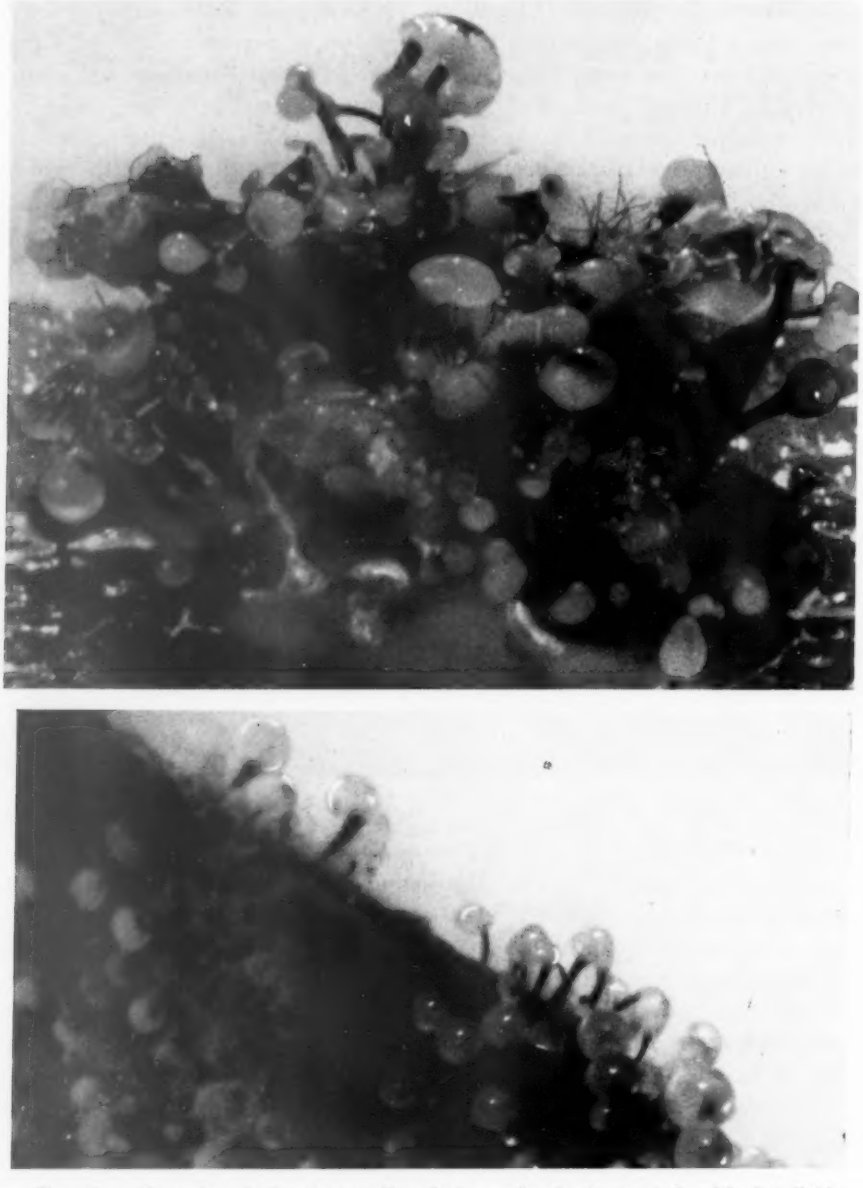

FIG. 2. Coremia of *Ceratostomella ulmi* on elm bark treated with 2,4-dichlorophenoxyacetic acid, photographed after 65 days: Upper photograph, abnormally large fasciated coremia and synnemata, other large immature forms, and numerous smaller coremia developing from yeast-like spore mass. Lower photograph, coremia of normal size and form. Photographs slightly retouched. 4×

ulmi, several groups of abnormally large coremia were observed. In all instances these abnormal growths were on sterilized elm bark that had been

placed in Petri dishes containing 10 to 15 cc. of a saturated solution of 2, 4-dichlorophenoxyacetic acid or the ammonium salt of this acid in concentrations varying from one to six grams per 800 cc. of sterilized water. Previous tests had indicated that stronger solutions inhibit the growth of *C. ulmi*. After the bark was inoculated with a spore suspension of *C. ulmi*, the cultures were incubated for 45 days or about three times longer than usually required for normal coremia production. The resulting groups of abnormal coremia varied from 7 to 20 mm. in diameter. These large

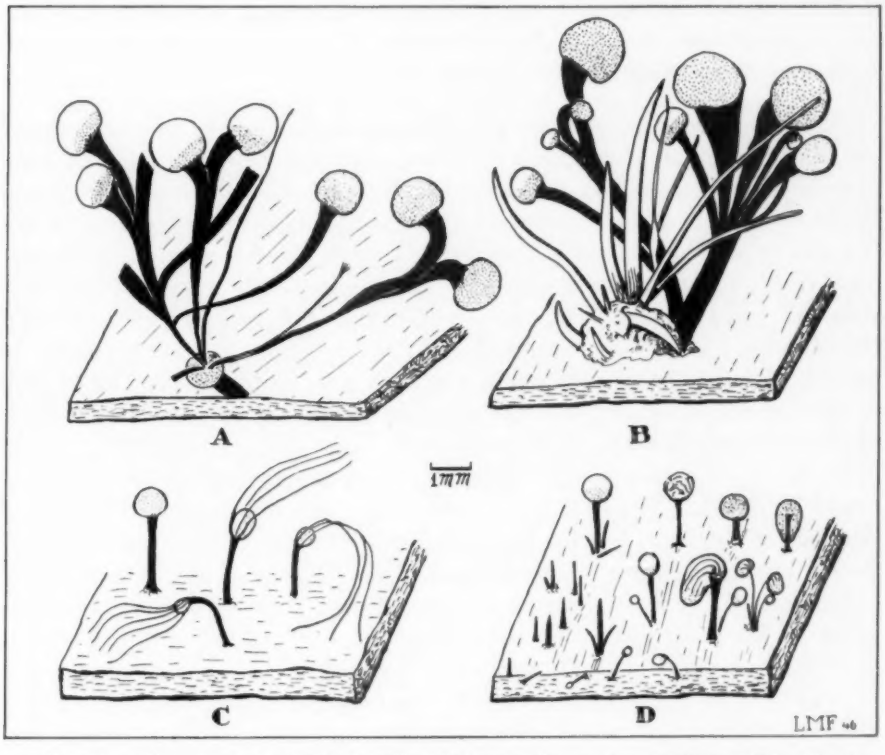

FIG. 3. Diagrams of *Ceratostomella ulmi* coremia on elm bark treated with 2,4-dichlorophenoxyacetic acid: A, Prostrate, fasciated coremia arising from coremial spore mass on bark surface; B, Upright group developed from yeast-like spore mass, and immature hyaline stalks as in figure 2, A; C, Primary coremia of normal length with filamentous, whiplike extensions; and D, Comparative size and form of normal mature and immature coremia on untreated elm bark in moist chamber.

overgrowths appear to have emerged from large masses of yellowish spore material on the surface of the inner bark. The sizes of the abnormally large coremial masses may be compared with those of a group of normal coremia in figure 2. These growths are illustrated diagrammatically in figure 3.

In the fasciated groups (Fig. 1) the individual coremia were 8 to 10 mm. in length from the base of the stalk to the top of the coremial head. The diameter or width of the tubular and flattened stalks ranged from 0.25

to 1 mm. Within the large coremial groups there were long, hair-like synnemata or dark brown filaments more or less than 3 mm. in length (Fig. 2). These hair-like filaments, which extended above the coremial head, seemed to originate from the top of the stalk. Their tips were hyaline and possibly fertile. On each flat, fasciated stalk a coremial head developed into a broadly elliptical mass of spores instead of the usual spherical mass. Subcultures from these abnormally large heads produced apparently normal mycelial colonies at the end of 20 days on potato-sucrose-agar.—LAWRENCE M. FENNER and LESTON R. FATE, Bureau of Entomology and Plant Quarantine, Agricultural Research Administration, United States Department of Agriculture, East Orange, New Jersey.

Cinchona Root Disease Caused by Phytophthora cinnamomi.[1]—Nurseries for the production of cinchona planting stock for experimental purposes have been maintained at the Estación Experimental Agrícola de Tingo María since early 1943, although it was recognized that the altitude (2,200 ft.) was below the generally accepted optimum for this species. Development of the plants has been somewhat faster than at the regular plantation sites, but, until the past year, diseases other than top die back[2] have not been troublesome. Root disease caused by *Phytophthora* has been absent or unnoticed until the attack herein reported was observed. At the height of the wet season of 1945–46, one-year-old transplants of the Ledger form of *Cinchona officinalis* L. developed chlorosis, progressively followed by loss of all but the terminal leaves on the branches and main stem and within a month by death. The first plants to develop symptoms were in the lower portions of the beds, but by the end of the wet season the disease had involved the entire nursery consisting of about fifteen 100-foot beds. Examination of the root systems of the diseased plants disclosed that the collar region and larger roots were in good condition, but that the smaller rootlets were brown and either dead or diseased.

Phytophthora cinnamomi Rands was isolated from almost all of the rootlets cultured. *P. cinnamomi* has been reported as causing cinchona root rot in British Malaya.[3] However *P. cinnamomi* does not appear to have been heretofore reported as a pathogen on cinchona in the Western Hemisphere.

The disease may be reproduced on healthy seedlings by inoculation only under conditions of high soil moisture. Isolates of *Phytophthora cinnamomi* from cinchona are indistinguishable from isolates from root rot of

[1] A contribution from the Estación Experimental Agrícola de Tingo María, Peru, a technical agricultural service organization for the Orient of Peru, operated jointly by the Dirección de Colonización y Asuntos Orientales, Ministry of Agriculture of Peru and by the Office of Foreign Agricultural Relations, U. S. Department of Agriculture. This study was made possible by funds provided through the U. S. Interdepartmental Committee on Scientific and Cultural Cooperation and funds from the Peruvian Government.

[2] Crandall, Bowen S. and William C. Davis. Phytophthora wilt and stem canker of cinchona. Phytopath. 35: 138–140. 1945.

[3] Thompson, A. Notes on plant diseases in 1939. Malayan Agr. Jour. 28: 400–407. 1940.

avocado, *Persea americana* Mill. from the same region, but cross inoculations have not been made to determine if strain differences exist.

This disease should not be confused with the root disease, which is one of the limiting factors in Peruvian cinchona plantations.[4] The *Phytophthora* causing this disease is distinct from *P. cinnamomi* and has been described as a new species, *Phytophthora quininea*.[5]—BOWEN S. CRANDALL, Pathologist, Office of Foreign Agricultural Relations, United States Department of Agriculture and Chief, Department of Plant Pathology and Entomology, Estación Experimental Agrícola de Tingo María.

The Occurrence of Monilinia seaveri on English Morello Cherry.—During a period of 17 years spent investigating fruit diseases in northwest Arkansas the writer frequently has observed the conidial stage of *Monilinia seaveri* (Rehm) Honey attacking the leaves and fruit of mature wild cherry (*Prunus serotina* Ehrh.) trees as well as producing a blight[1] of the seedlings.

In spite of the widespread distribution of the fungus on this wild host, repeated examinations of cultivated plums and cherries in the vicinity of infected wild-cherry trees have failed, except in the two instances described in this note, to reveal infections of cultivated species by this fungus.

On May 8, 1934, one immature cherry on an English Morello (*Prunus cerasus* L.) tree at the University of Arkansas farm north of Fayetteville, Ark., was observed to be infected with a *Monilinia* decidedly different in appearance from the imperfect stage of the common brown-rot fungus, *Monilinia fructicola* (Winter) Honey.

A microscopic examination of the fungus on this single specimen showed conidia and disjunctors identical in appearance with those of *Monilinia seaveri*. The specimen likewise had the characteristic sweetish odor regularly associated with the presence of *M. seaveri* on wild-cherry trees.

No additional specimens were observed in 1934, but on April 29, 1935 the same fungus was found attacking the small, immature fruit on a number of English Morello cherry trees at the University farm. The common brown-rot fungus was also present on other fruit and the two fungi could be distinguished readily in the field. The conidia of *Monilinia fructicola* were present as cushion-shaped masses, while those of *M. seaveri* were closely appressed to the surface of the fruit, as may be seen in figure 1.

No attempts were made to infect wild-cherry leaves with conidia from the English Morello fruit. However, the marked similarity in appearance of the spore masses on the two hosts and the presence of the characteristic sweetish odor on the English Morello fruit, coupled with a microscopic study of spores and disjunctors from both hosts, indicate that the English Morello fruit was attacked by *Monilinia seaveri*.

[4] Crandall, Bowen S., and William C. Davis. Occurrence of Cinchona root rots in the Americas. U. S. Dept. Agr., Plt. Dis. Reptr. **28**: 926–929. 1944.

[5] Crandall, Bowen S. A new Phytophthora causing root and collar rot of cinchona in Peru. Mycologia **39**: 218–223. 1947.

[1] Dunegan, J. C. A blight of wild cherry seedlings. Phytopath. **30**: 89–90. 1940.

FIG. 1. Immature English Morello cherries partially covered with masses of conidia of *Monilinia seaveri*. The two cherries on the left, infected by *M. fructicola*, are included for comparison.

This natural infection of a cultivated host by *Monilinia seaveri* occurred during periods of excessive rainfall, which favored the profuse development of fungus diseases. Similar environmental conditions occurred in northwest Arkansas in 1939 and 1945, but no further cases of the infection of cultivated hosts by *M. seaveri* have been observed.—JOHN C. DUNEGAN, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture, Plant Industry Station, Beltsville, Maryland, cooperating with the Arkansas Agricultural Experimental Station.

Thelephora terrestris on Blueberry Plants.—The smothering or strangling fungus *Thelephora terrestris* Fr. (*T. laciniata* Fr.) has been reported as causing injury to forest tree seedlings, especially to conifers. The damage recorded was due to the covering or enveloping of the small seedlings in propagating beds by the fruiting structures of the fungus.

Weir[1] reported the occurrence of the fungus in the United States on *Abies, Picea, Pinus, Pseudotsuga,* and *Thuja* of coniferous forms, and on *Acer, Quercus,* and *Ribes.* All these reports of occurrence of *Thelephora terrestris,* except that for *Ribes,* came from western and central States.

In November, 1946, the writer observed the fungus[2] on 1-year-old blueberry (*Vaccinium australe* Small) nursery plants near Toms River, N. J. This is believed to be the first report of *Thelephora terrestris* in association with *Vaccinium.*

The nursery where the fungus was found was planted in inadequately drained sandy virgin soil and contained considerable muck and other unde-

[1] Weir, James R. *Thelephora terrestris, T. fimbriata,* and *T. caryophyllea* on forest tree seedlings. Phytopath. 11: 141–144. 1921.

[2] John A. Stevenson, Div. Mycology and Disease Survey, identified the fungus as *Thelephora terrestris* Fr.

composed plant material. The plants were propagated from cuttings made in 1945 and had grown one year in the nursery rows. The plants were small and well spaced and produced very little shade to favor the growth of the fungus. Although no test was made of the pH of the soil, it undoubtedly was low as blueberries thrive best in acid soils. The fungus was attached to about 2 dozen plants, all confined to an area of about 20 square feet on one side of the nursery and adjacent to uncleared land.

The fungus structures, surrounding the plant stems at and immediately below the ground level, were partly exposed and on account of their large size were easily seen. They varied from 1 to 2 inches in diameter. Figure 1

FIG. 1. *Thelephora terrestris* on a 1-year-old blueberry plant. Toms River, N. J. (× 1.)

is a photograph showing a fungus fruiting body about natural size, on a blueberry plant, after it had been thoroughly cleaned. When the fungus was collected the size of the body was even more impressive, as in addition to the fungus structure there was a considerable amount of soil and plant fragments all tied together in a compact mass by hyphae.

The fungus was loosely attached to the plants and could easily be broken off. There was no evidence of injury done except to some leaves that were in contact with the soil. Those leaves were completely covered with the fungus and a few were dead and had abscised.—J. B. DEMAREE, Plant Industry Station, Beltsville, Maryland.

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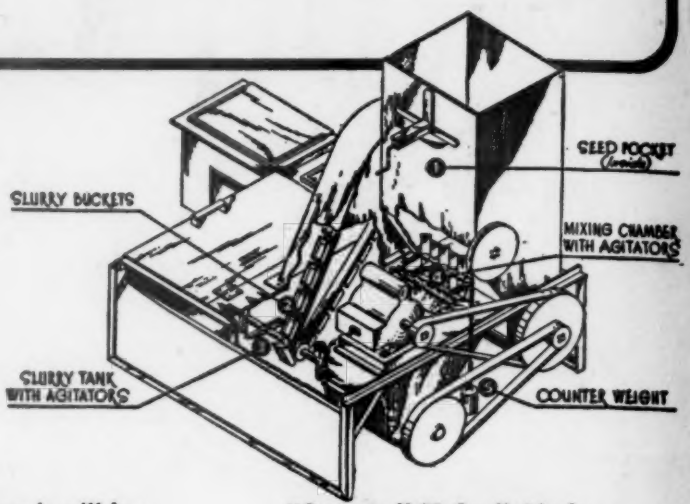

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